



# *The Journal of Laboratory and Clinical Medicine*

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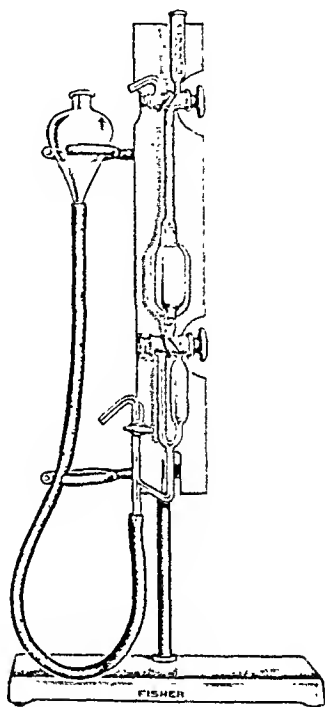
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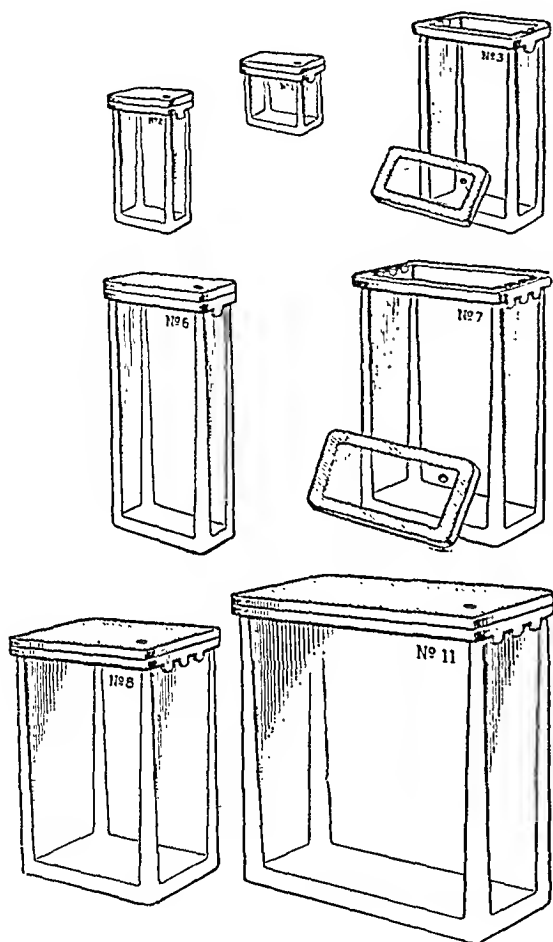
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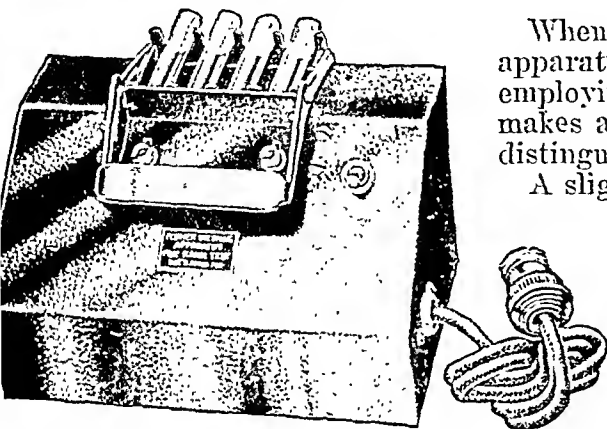
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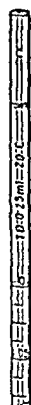


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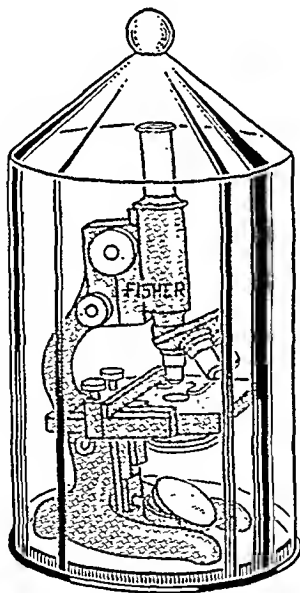
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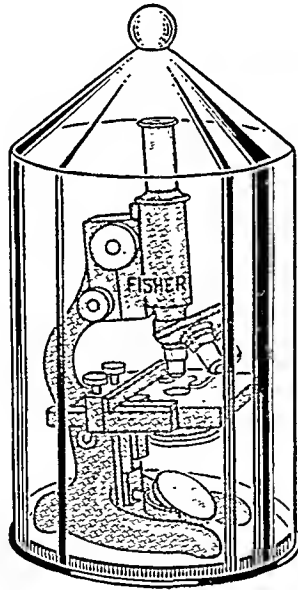
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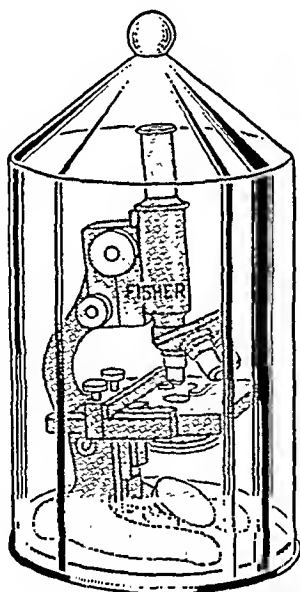
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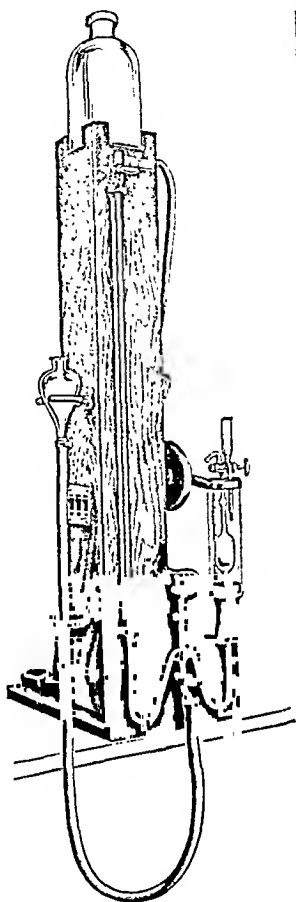
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# *The Journal of Laboratory and Clinical Medicine*

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VOL. 20

OCTOBER, 1934

No. 1

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## *CLINICAL AND EXPERIMENTAL*

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### VARIATIONS IN REACTION OF DIFFERENT PARTS OF THE CENTRAL NERVOUS SYSTEM AS INFLUENCED BY DEPRESSANT AND STIMULATING DRUGS\*

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D. E. JACKSON, PH.D., M.D., CINCINNATI, OHIO

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NEARLY forty years ago Cushny emphasized the fact that if two animals be poisoned, one with morphine, the other with chloral to a degree which would just prevent the appearance of spontaneous movements in each, then the spinal reflexes of the animal which had received the morphine would be much more active and more easily elicited than would be the case with those of the animal under the influence of the chloral. It seems certain that some such similar variations as this extend throughout the central nervous system whenever the action of any two drugs which act thereon are compared. My attention was again especially attracted to this phenomenon many months ago when I was observing the action of certain drugs on the respiratory center.

This is a very old subject in pharmacology and a great many papers bearing on one or more phases of the problem have appeared in the literature. It is impossible for me to cite all of these here but I may refer briefly to the excellent papers by Tatum, Waters, Maloney and Fitch (bibliography),<sup>1</sup> Camp,<sup>2</sup> Barlow<sup>3</sup> and his colleagues, Nielsen, Higgins and Spruth,<sup>4</sup> Swanson<sup>5</sup> and his coworkers, Behrens and Reichelt,<sup>6</sup> and Eddy.<sup>7</sup>

The specific depressant action of morphine on the respiratory center has long been known. But one does not ordinarily think of such drugs as ether or chloroform as possessing this specific action, because the depression pro-

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\*From the department of Pharmacology of the University of Cincinnati Medical School.



duced by these drugs appears to be so evenly distributed over the whole central nervous system. On the other hand the tendency of morphine to mildly excite the cord centers and a few higher nuclei (markedly developed in the

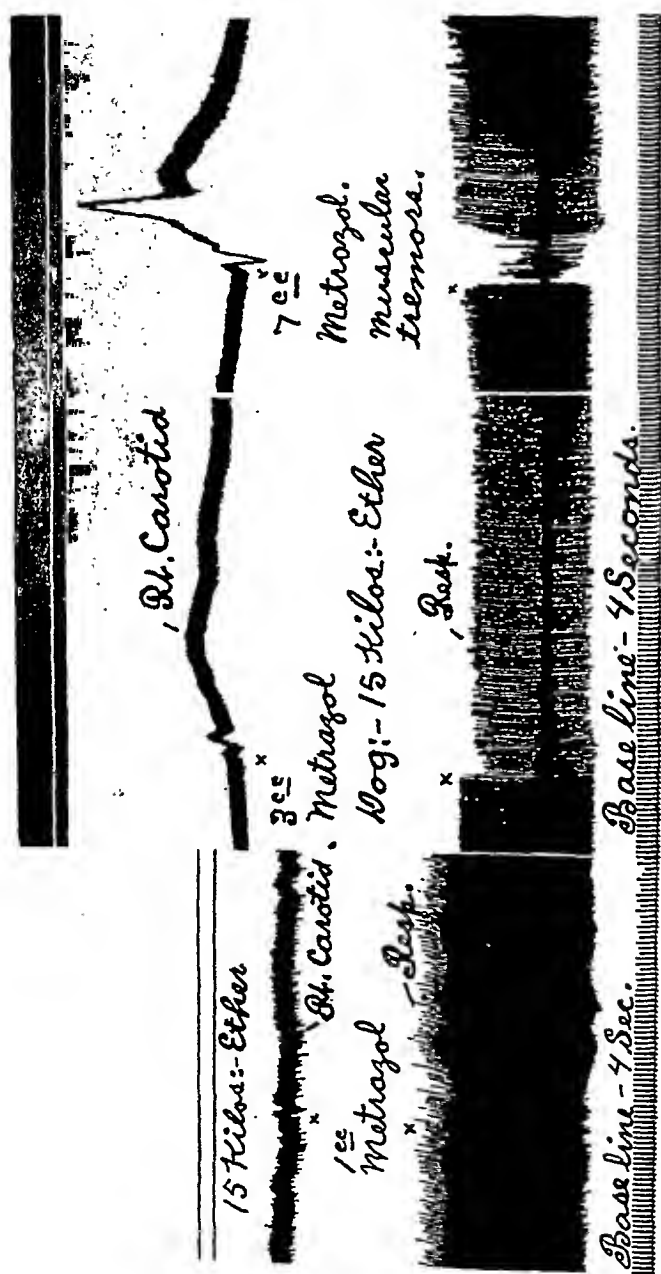


Fig. 1.—For discussion see text. This illustration is made up of three sections all taken consecutively from the same experiment and mounted together. They show the effects of increasing dosage of metrazol on an etherized dog.

felines and in frogs in the second stage of poisoning) is practically never thought of in connection with the pharmacologic action of ether or chloroform. Nevertheless these varying degrees of stimulation and depression of all the various centers and tracts of the whole brain and cord are undoubtedly

always present when drugs are acting on the central nervous system. A year or so ago I became impressed with the therapeutic importance of an example of this type of reaction which I observed experimentally in the laboratory.

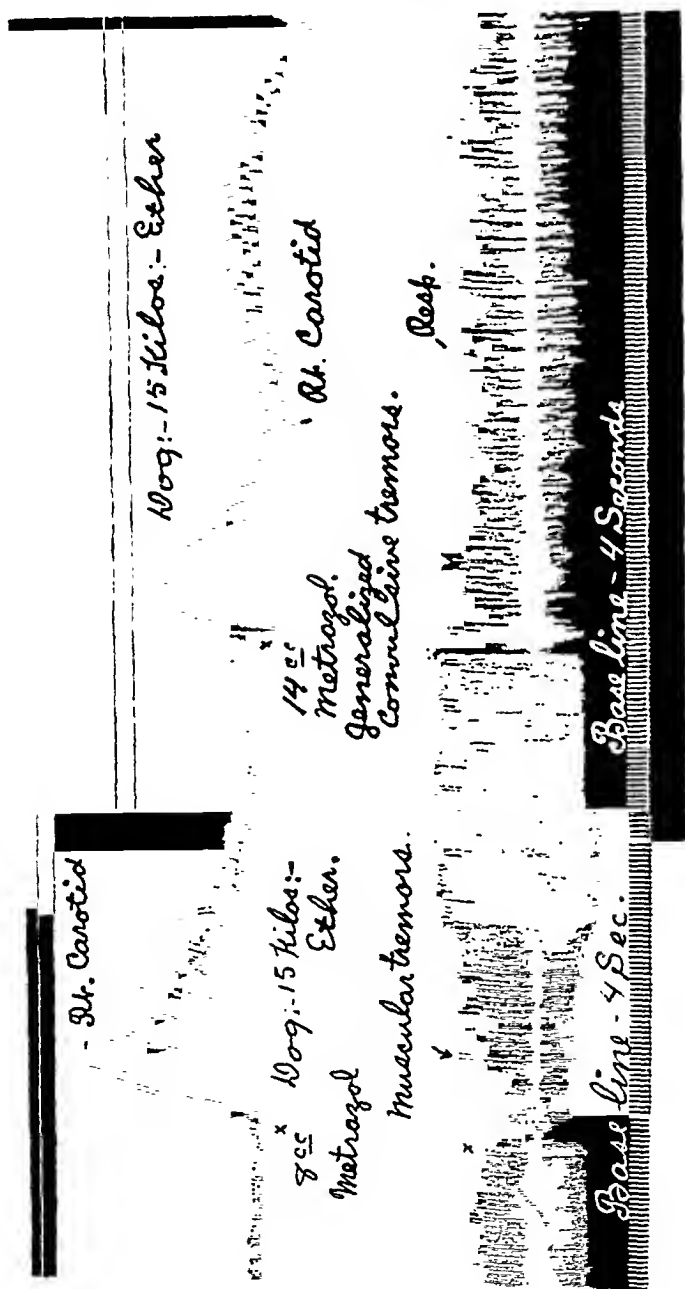


Fig. 2.—Two sections of tracings from the same experiment mounted together. For discussion see text.

This was the failure of metrazol (cardiazol) to produce any worth-while stimulation of the respiratory center in dogs when deeply anesthetized with ether. This observation had also been reported earlier by Camp who obtained similar results with chloroform and morphine. On the other hand Camp and

many others have noted the fact that fair-sized doses of metrazol produce convulsions in animals deeply anesthetized with ether.

All of the experiments described in this paper have been carried out on dogs. I have tried many times to see if I could produce any therapeutically useful stimulation of the respiratory center with metrazol in dogs anesthetized with ether. The lighter the anesthesia the more likely is the metrazol to pro-

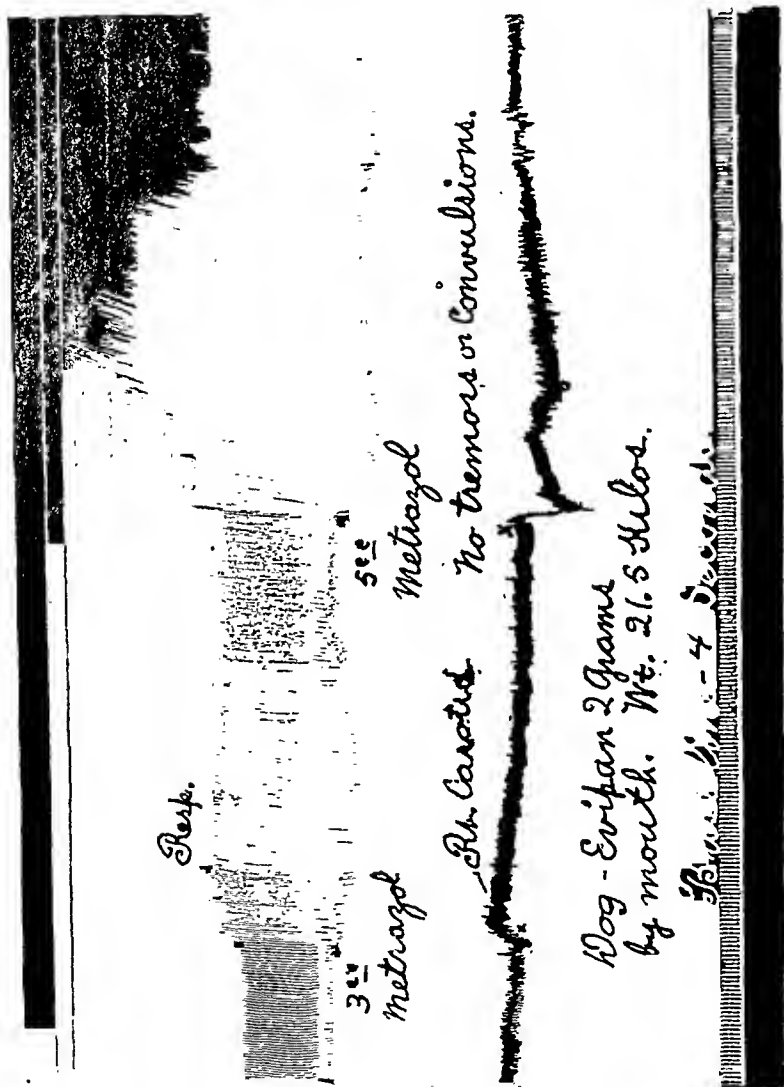


FIG. 3.—This tracing shows the action of metrazol on a dog anesthetized with evipal (evipal).

duce obvious results. And with a very light anesthesia I have occasionally seen a mild respiratory stimulation produced by metrazol. But as the anesthesia is deepened the likelihood that metrazol, if injected intravenously, will specifically stimulate the respiratory center rapidly disappears, and only the probability remains that adequate doses will produce tremors or twitchings or generalized convulsions which appear to come from both the medulla and the cord (Figs. 1 and 2). This would seem to render metrazol useless in many

instances in which a respiratory stimulant is most needed. Two questions which I have not been able to answer satisfactorily arise in this connection, viz., first, does metrazol act (or fail to act) on the human respiratory center when it is deeply depressed by ether in the same way that the drug acts on the dog respiratory center under similar circumstances, and second, if a failing patient continues to breathe feebly after administration of ether has been

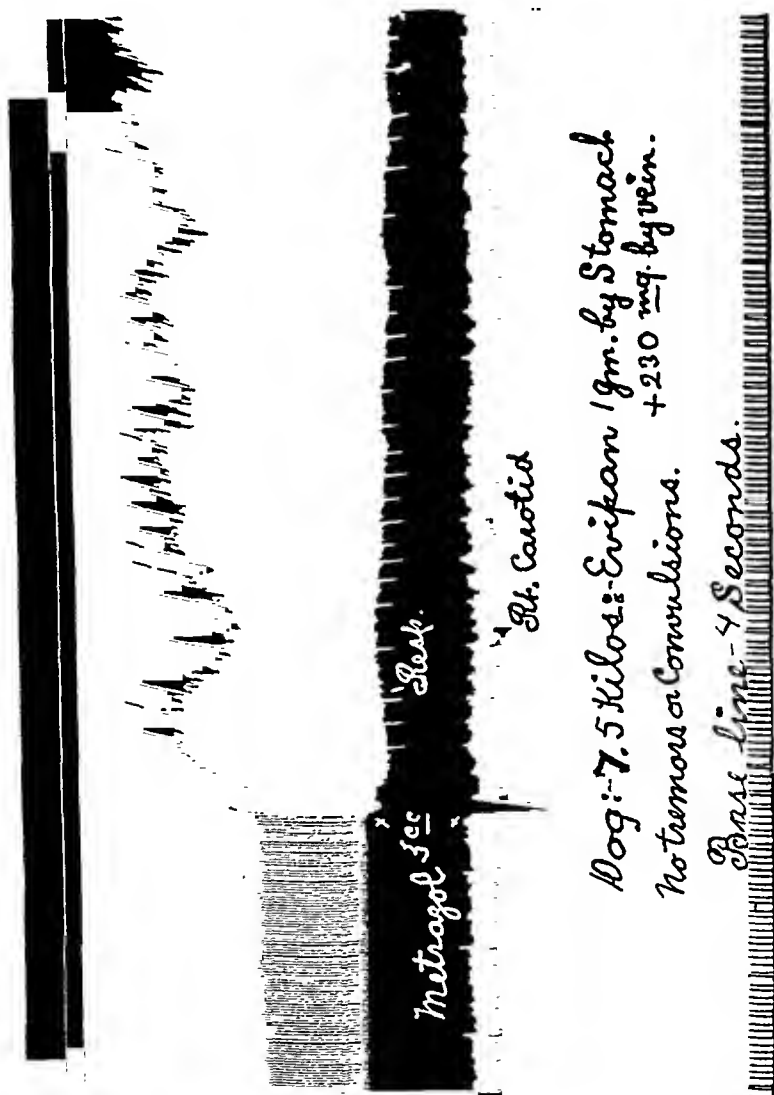


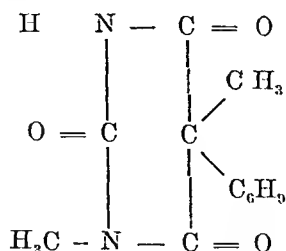
Fig. 4.—Metrazol following evipan.

stopped, will the ether tension in the patient's blood and medulla fall low enough, within reasonable limits of time, to allow the metrazol to exert a therapeutically useful effect on the center? For the present, these questions must go unanswered, but I suspect that the ultimate answer will be "yes" in both instances.

Here I should perhaps emphasize that, in the light of comparison with experiments described below, ether appears to possess a specific depressant

action on the respiratory center. For metrazol tends to produce tremors and convulsions, rather than respiratory stimulation, in dogs that are fully anesthetized with ether. And this is not simply a matter of the size of the dose, for a dose of metrazol of sufficient size to produce in an etherized dog convulsions only, with no efficient stimulation of the respiratory center, will produce a marked specific stimulation of the respiratory center with no vestige of tremors or convulsions in a dog which has been comparably anesthetized with evipal (see Fig. 3).

The contrast between the actions of evipal (evipan) and metrazol on the respiratory center are further illustrated in Fig. 4. This tracing was taken from a small dog (7.5 kilos) yet 3 c.c. (4.5 grains) of metrazol produced profound, vigorous, and rapid respiration by a direct stimulation of the respiratory center, while no tremors or convulsions whatever could be observed. The slow and rather weak character of the respiration previous to the injection of the metrazol shows clearly that the animal was deeply anesthetized by the evipal (evipan). The action of evipal (the new name for evipan) is of shorter duration than that of most of the older hypnotic drugs. Hence in this experiment a solution of evipal was kept at hand and small additional doses were injected from a buret intravenously from time to time in order to hold the anesthesia at any depth desired. An original dose of one gram dissolved in water and given by stomach tube produced a deep, quiet anesthesia in this dog (7.5 kilos) in five or six minutes. This drug may be very useful for experimental purposes, for by occasional small intravenous injections of later doses of the drug, the anesthesia can be kept at almost any desired level, and the specific counteracting effect of metrazol on the respiratory center can be used to revive the animal and arouse it almost to a state of consciousness. This metrazol stimulation, however, can be at once counteracted by an additional dose of evipal. This counteracting effect of the two drugs may be repeated many times during the course of a long experiment (see Fig. 5, nembutal). Evipal is N-methyl-cyclohexenyl-methyl-malonyl-urea having the following formula:



The sodium salt is readily soluble in water but is unstable and the powder should be freshly dissolved in water immediately before injection (by stomach tube, intravenously or intraperitoneally).

The striking antagonistic actions of metrazol and nembutal are well shown in Fig. 5. This animal was deeply anesthetized with nembutal and at the beginning of the tracing 8 c.c. of metrazol were injected by femoral vein. The

respiratory center was promptly stimulated but following this 2 gr. of nembutal were injected and this immediately depressed the respiration. There was a sharp temporary fall in blood pressure following each of these injections.

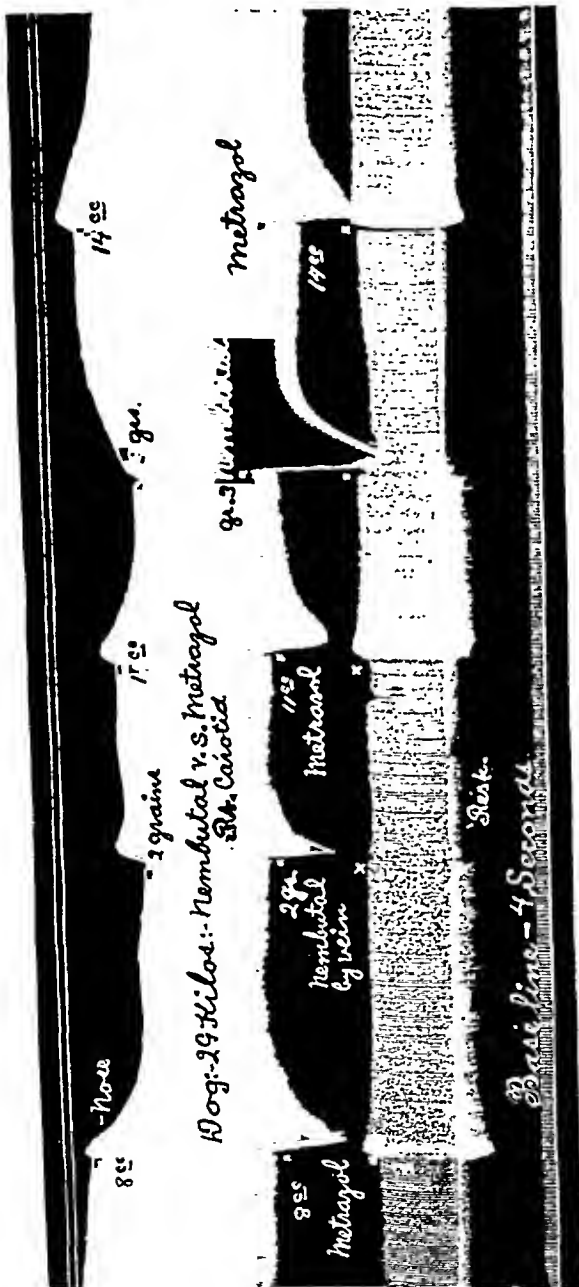


Fig. 5.—Five injections showing the alternate opposing actions of metrazol and nembutal.

This type of vascular reaction is common to a great many substances and its cause (or causes) is not well understood. It may be due to the sudden application to the endocardium of a rather strong solution of the drug (Abel), or to a similar sudden application of the solution to the medullary or lower

centers, or possibly to the lining of the arterioles, etc. These temporary blood pressure falls are of no especial significance, but there is one point which

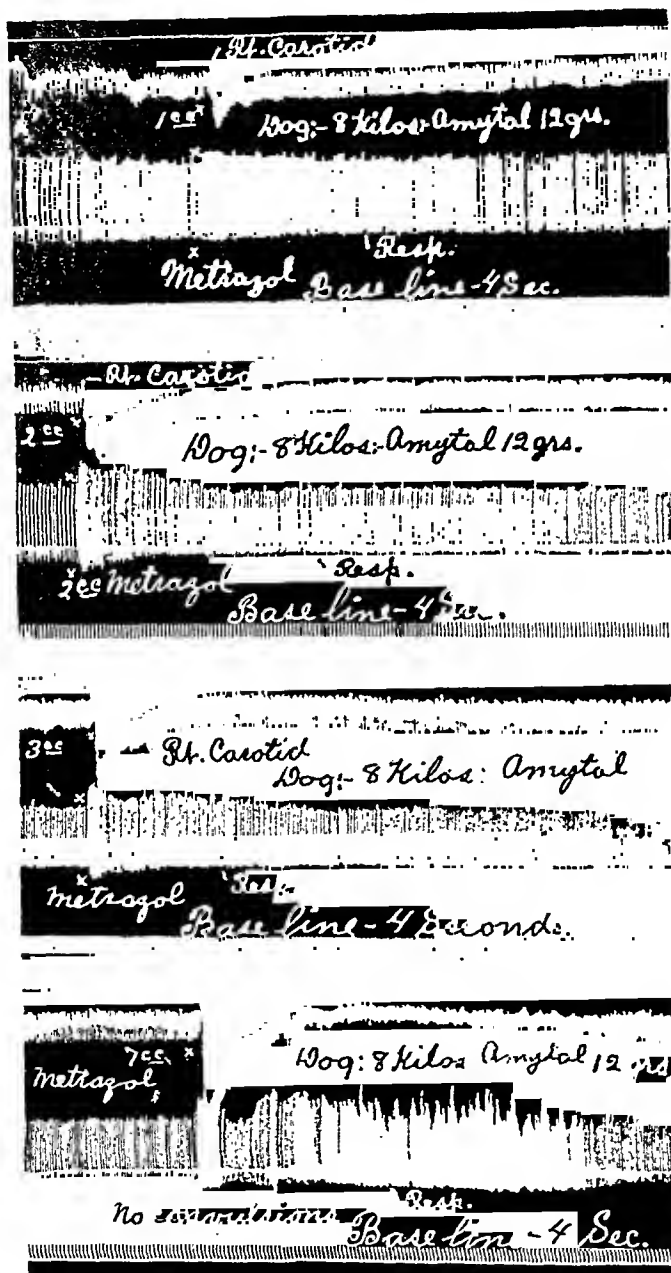


Fig. 6.—Four sections from the same experiment mounted consecutively from top to bottom and showing the effect of increasing dosage of metrazol on a dog anesthetized with amytal.

should be kept in mind in experiments of this type, viz., that a sudden, sharp fall in blood pressure may produce sufficient anemia, and hence asphyxia, in the medulla to act as a stimulation on the respiratory center. Since the first

fall in pressure (metrazol) in this tracing was accompanied by a stimulation of the respiration, and the second fall (nembutal) by a depression of the respiration it seems obvious that the blood pressure changes had little or no

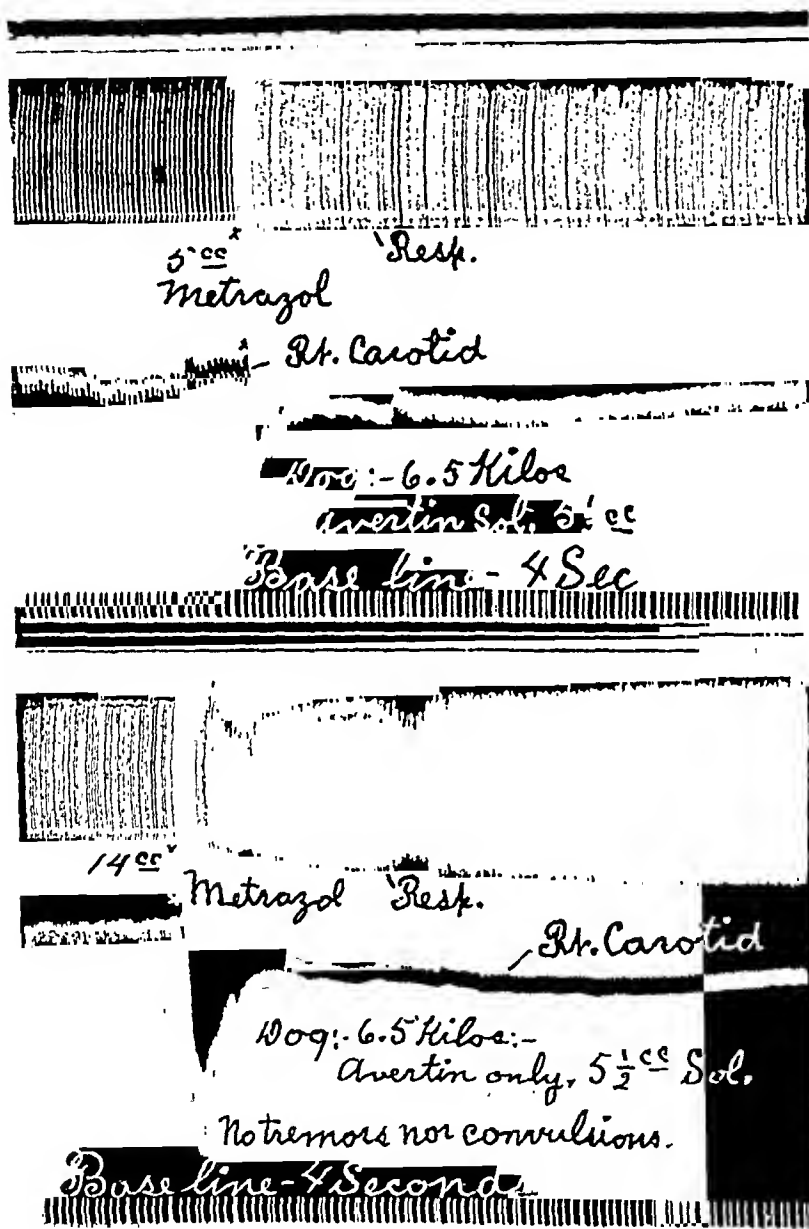


Fig. 7.—Two sections from the same experiment mounted consecutively from top to bottom and showing the effect of increasing dosage of metrazol on a dog anesthetized with avertin.

effect on the respiration. The three subsequent injections of metrazol, nembutal, and metrazol show the counteracting effects of these two drugs on the respiratory center.



Attention should be called to the matter of dosage in these experiments. Dogs are much less sensitive to these drugs than are human beings, so that while a dose of metrazol which is apparently out of all proportion to the amount which could be given to a patient may be injected into a dog, still

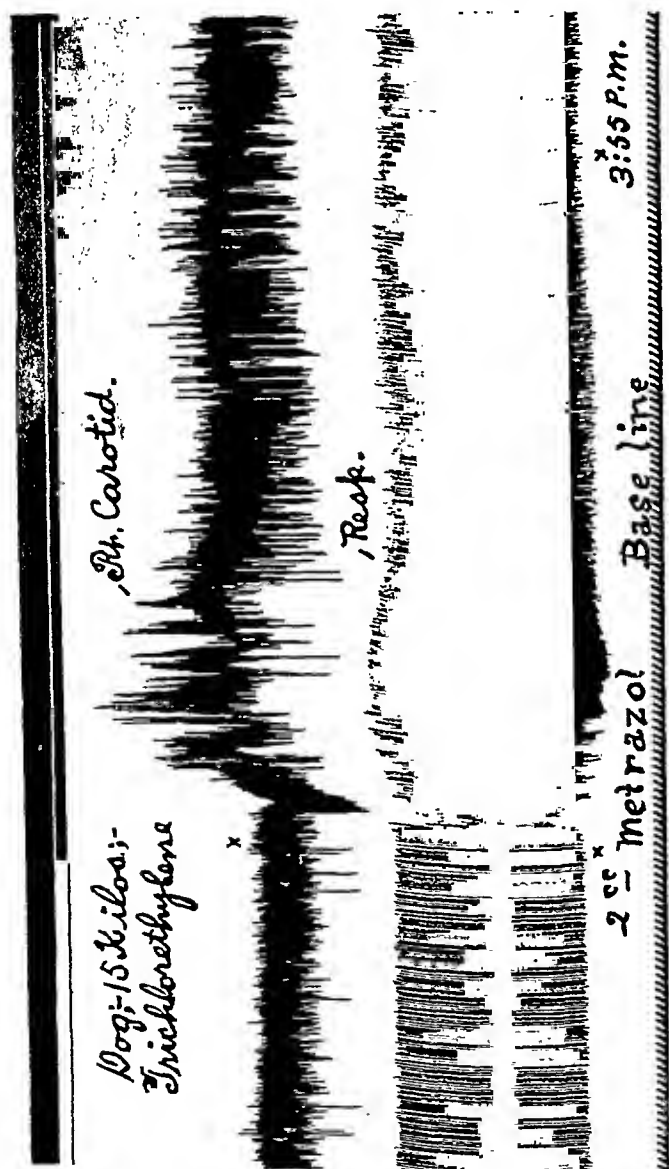


Fig. 8.—Tracing showing the effects of metrazol on a dog anesthetized with trichlorethylene.

it should also be noted that a correspondingly large increase in the size of the dose of the hypnotic has been required to produce the anesthesia. And the specific opposing actions of such drugs as evipal and metrazol on the respiratory center seems to be so definite and clear cut that, after the first injection of each drug (to test the individual sensitivity of the animal), one can thereafter predict with a high degree of accuracy the exact size of the

dose of each drug which will be needed just to counteract the effects of the other. It must be held in mind, of course, that, within the limits of tolerance, the animal becomes progressively less and less susceptible to each succeeding dose of a given drug, so that the amount injected each time must be progressively increased to make up for this loss in susceptibility.

A very similar counteracting action on the respiratory center is manifested by amytal and metrazol (Fig. 6) and by avertin and metrazol (Fig. 7).

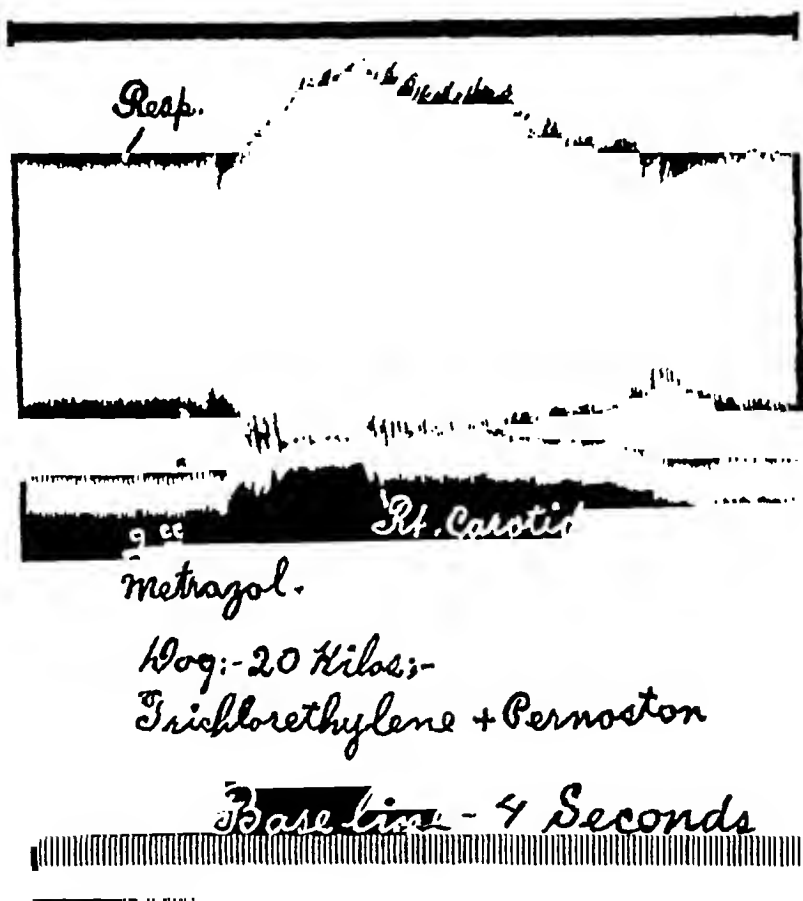


Fig. 9.—Tracing showing the effects of metrazol on a dog anesthetized with trichlorethylene and pernoston. Note the rise in blood pressure due mainly to contraction of the splanchnic blood vessels.

This shows that these drugs of widely differing chemical structure still differ quite markedly from ether in the action which they manifest on the respiratory center and on the other centers of the medulla and the spinal cord. For huge doses of metrazol are unlikely to produce convulsions in animals deeply anesthetized by amytal or avertin (as would happen under ether) but on the other hand metrazol markedly and specifically stimulates the respiratory center in animals anesthetized by amytal or by avertin, but has little or no specific respiratory stimulating action in animals anesthetized by ether.

It is extremely probable that all drugs which stimulate the respiratory center (or any other part of the central nervous system) could be shown to manifest some such varying reactions when administered to animals anesthetized by a series of different chemical substances. And it is probable that these variations might often be of therapeutic importance. For this particular series of experiments metrazol has simply served as a convenient agent to reveal and emphasize a few facts which are ordinarily obscured by the soothing influence of a deep sleep.

Fig. 8 shows the action of metrazol on a dog anesthetized with trichlorethylene. The type of anesthesia produced by trichlorethylene is different from those produced by either the hypnotics or ether. Yet it can be seen from this tracing that metrazol stimulates the respiratory center in dogs anesthetized with trichlorethylene. It appears that the anesthesia produced by this drug resembles in varying degrees the anesthetics produced by both ether and the hypnotics. For here metrazol stimulates both the respiratory center and, to a less extent, the reflexes of the cord and perhaps of the medulla. The metrazol tends to produce tremors and mild convulsive movements, as well as a marked increase in respiratory rate.

Fig. 9 shows the influence of metrazol on a dog anesthetized by both trichlorethylene and pernocton. Here the respiration is again markedly stimulated but there is considerably less tendency for the development of tremors and convulsive twitching. Apparently the (cord and medulla) stimulating action of the metrazol has been efficiently counteracted by the pernocton. And here a small dose (2 c.c.) of metrazol produced a marked respiratory stimulation in a large (20 kilos) dog.

Experimentally it seems that unanesthetized dogs are very much more sensitive to metrazol than is the case when the animal is under the influence of any of the central nervous depressing drugs whatever. And I suspect that generalized tremors or convulsions, without specific respiratory stimulation, are rather more likely to occur if no anesthetic substance is used. Whether similar reactions would occur in man in the absence of a central nervous depressant I cannot say. But this point might be of considerable interest clinically in the treatment of shock from trauma, drowning, poisoning, etc. From the varying reports of the therapeutic usefulness of metrazol in the literature, ranging from excellent to questionable, I suspect that the drug is very efficient in the proper cases, but inefficient in others.

#### CONCLUSIONS

1. No two general anesthetics, or hypnotics, possess the same action on the central nervous system. When the anesthesia produced by each of two substances appears to be exactly the same, a marked difference may frequently be shown if a substance having a specific stimulating action on some particular part or center of the central nervous system be injected to counteract the anesthesia.

2. In a dog anesthetized with ether, metrazol produces marked generalized tremors or convulsions, but with little or no specific stimulation of the respiratory center.

3. In a dog deeply anesthetized with aripal (and many other of the more recent hypnotics), metrazol produces marked specific stimulation of the respiratory center but without any symptoms of tremors or convulsions.

4. In a dog anesthetized with trichlorethylene, metrazol produces a combination of these reactions. The respiratory center is stimulated and some tremors and convulsive twitchings are produced.

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## THE MECHANISM OF THE BLOOD CHANGES DURING THE TREATMENT OF SECONDARY AND PERNICIOUS ANEMIA\*

C. A. ELVEHJEM, PH.D., AND M. O. SCHULTZE, M.S., MADISON, WIS.

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THE fact that reticulocyte responses very similar in nature have been observed during the treatment of both pernicious and secondary anemias introduces an interesting question concerning the mechanism of these blood changes. Minot and Heath<sup>1</sup> have presented examples of the course taken by the reticulocytes in response to potent liver preparations in pernicious anemia and in response to maximal amounts of iron in anemia chiefly due to chronic blood loss. In general, the responses are very similar except that those in the case of secondary anemia are more prolonged.

Schultze and Elvehjem<sup>2</sup> have recently demonstrated that a typical reticulocyte response in anemic rats can be obtained only when adequate amounts of both iron and copper are fed, and they suggest that the initiation of reticulocytosis is dependent upon newly formed hemoglobin and that the subsequent decrease in the number of these cells is influenced by the amount of hemoglobin present in the blood. When suboptimal amounts of either iron or copper were fed, a much prolonged response occurred. In the light of these facts, the results

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\*From the Department of Agricultural Chemistry, University of Wisconsin.  
Received for publication, January 5, 1934.

recorded by Minot and Heath<sup>1</sup> in secondary anemias may have been affected by an insufficient supply of copper.

A true explanation of the rapid changes in the number of reticulocytes in the blood during the treatment of anemias will depend to a large extent upon histologic studies of the hematopoietic organs which produce these cells—mainly the bone marrow. This, of course, is rather difficult in human patients. The histologic changes need to be studied daily in order that they may be correlated with the blood changes. In the case of secondary anemia such studies can now be made with rats. In the meantime we wish to suggest the following explanation of the changes observed in the blood during the treatment of pernicious and secondary anemias.

In order to simplify the discussion, the deficiency in pernicious anemia will be considered as a lack of ability to form red cells and the deficiency in secondary anemia a lack of adequate hemoglobin-building power. The bone marrow will be considered as the center for red cell formation and the liver the centralized organ for hemoglobin production. There may be some criticism of the latter assumption but this will not affect the discussion. An adequate supply of the P.A. factor will be considered positive treatment for pernicious anemia and an adequate supply of iron and copper positive treatment for secondary hypochromic anemia. No attempt will be made to discuss the source or methods of supplying these materials or the more complicated forms of anemia which are encountered.

A condensed outline of the changes taking place in the liver, bone marrow, and blood during the development and treatment of secondary and per-

TABLE I

CHANGES OCCURRING DURING THE DEVELOPMENT AND TREATMENT OF SECONDARY AND PERNICIOUS ANEMIAS

STAGE	TISSUE	SECONDARY	PERNICIOUS
Development	Liver	Deficiency of iron and/or copper. No Hb. formation	Sufficient iron and copper. Hemosiderosis
	Bone marrow	P. A. factor available	Deficiency of P. A. factor. Fragile cells produced. Exhaustion
	Blood	Decrease in total Hb. Decrease in Hb. per cell Decrease in no. of red cells	Decrease in no. of red cells. Increase in Hb. per cell. Decrease in Hb.
Treatment with suboptimal amounts of iron and/or copper (secondary anemia) or small amounts of P.A. factor (pernicious anemia)	Liver	Some Hb. formation	
	Bone marrow	Hb. stimulates cell formation	Slow cell formation
	Blood	Increase in reticulocytes. Insufficient Hb. to form normal cells. Hb. and cells low. Reticulocytes high	Prolonged reticulocyte response. Small increase in cells and hemoglobin
Treatment with adequate amounts of iron and copper (secondary anemia) or optimum amounts of P.A. factor (pernicious anemia)	Liver	Rapid Hb. formation	Hemoglobin formation if sufficient Fe and Cu are present
	Bone marrow	Rapid cell formation	Rapid cell formation
	Blood	Typical reticulocyte response. Increase in Hb. and red cells	Typical reticulocyte response. Increase in cells and Hb.

nicious anemias is given in Table 1. In secondary anemia there is a deficiency of iron and/or copper due either to a deficient supply from the food or to a too rapid utilization of the stores of these elements in the body. When these elements are no longer available, the liver is unable to build sufficient hemoglobin, and consequently, there is a decrease in the total hemoglobin of the blood. For a time the cells continue to be produced, which fact causes a decrease in the amount of hemoglobin per unit of cell. The new cells which are formed cannot be supplied with hemoglobin and they are destroyed by the reticuloendothelial system. The bone marrow soon gives up its attempt to manufacture cells and a condition of aplasia develops. McGowan<sup>3</sup> has observed an aplastic condition in the bone marrow of severely anemic pigs. The ordinary marrow elements were present, but there was an absence of signs of activity and the cells were greatly reduced in numbers.

When suboptimal amounts of iron and/or copper are supplied in this type of anemia, a small amount of hemoglobin is produced immediately. The presence of this newly formed hemoglobin (or a possible precursor) stimulates the bone marrow to rapid cell formation and causes a very definite increase in the number of reticulated cells in the circulating blood. The quantity of hemoglobin formed in this case is limited and the new cells do not receive a normal supply of pigment. These cells are destroyed and the stimulus for new cell formation continues. Under such conditions a prolonged reticulocyte response similar to that described by Schultze and Elvehjem<sup>2</sup> is obtained.

If adequate amounts of iron and copper are available, the picture is quite different. Rapid hemoglobin formation takes place in the liver. The newly formed hemoglobin stimulates cell formation and the bone marrow pours the reticulocytes into the blood. Now the new cells are supplied with sufficient hemoglobin; they mature into normal red cells, and the impetus for further cell formation is reduced. In a short time the hemoglobin and red cells approximate normal values and the number of reticulocytes decreases because only a small number of new cells need to be formed. The greatest reticulocyte response occurs when the hemoglobin and red cells are the lowest because there is then a greater requirement for new cells. Cell formation is not complicated by an insufficiency of stroma building material in true secondary anemia because there is plenty of the P.A. factor available.

In pernicious anemia the condition is quite different, here hemoglobin can be formed, but the bone marrow is unable to produce cells due to the absence of the P.A. factor. The cells which are formed are fragile and are destroyed immediately. The stimulus for cell production is present and the marrow attempts to produce cells until it becomes completely exhausted. Since hemoglobin can be formed, the cells present in the blood carry an increased amount of pigment. When the cells are destroyed, the hemoglobin cannot be carried by the blood and hemosiderosis takes place due to the accumulation of the iron set free from the destroyed cells.

When small amounts of the P.A. factor are supplied, the bone marrow produces new cells slowly, the reticulocyte response will be small and prolonged, and the hemoglobin and red cells increase only slightly. In the presence of optimum amounts the new cells are formed rapidly and a decided increase in the reticulocytes is observed. There is sufficient hemoglobin to supply

the new cells and the mature cells are readily formed. The impetus for cell formation decreases and a corresponding decrease in reticulocytes follows. The reticulocyte response may be prolonged if there is not sufficient iron and copper present for rapid hemoglobin formation.

This explanation is very brief and general in character, but we hope it will aid in formulating certain fundamental principles necessary for a more complete understanding of anemia.

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## THE EFFECT OF ULTRAVIOLET LIGHT ON SOME SYMPATHOMIMETIC SUBSTANCES\*

PAUL L. EWING, M.S., CHICAGO, ILL.

THE action of ultraviolet light on drugs presents an insight into the changes that occur in such drugs in everyday experience when they are exposed to ordinary daylight. The photochemical action of light is chiefly due to the ultraviolet region; consequently, exposure to the rays of a quartz mercury arc lamp for a short time corresponds to a long exposure to daylight. This investigation is a continuation of work previously published.<sup>1</sup>

Relatively little work has been done on this particular problem. Savopol,<sup>15, 16, 17</sup> Verda, Kneer, and Burge,<sup>20</sup> Kneer, Orth, Verda and Burge,<sup>6</sup> and Ito and Terata<sup>5</sup> have reported on the effect of ultraviolet irradiation of solutions of epinephrine. The action of these rays on other drugs has been reported by a few investigators: Pacini and McGuigan,<sup>11</sup> Wakeham and Tracy,<sup>21</sup> Higgins, Ewing, and McGuigan,<sup>3</sup> and Gant<sup>2</sup> on nicotine; Macht<sup>9</sup> on digitalis. In the majority of instances, destruction of the drug is found. As was shown in our previous paper,<sup>1</sup> this may not always be the case.

This study is limited to three sympathomimetic drugs—epinephrine (adrenalin—Parke, Davis and Company), synephrin (Frederick Stearns and Company, N. N. R., 1931), and p-hydroxyphenyl-1-amino-2-propanol-1 (Sharp and Dohme).

#### METHODS

**Ultraviolet Irradiation.**—From 10 to 30 c.c. of 0.01 per cent epinephrine, 0.1 per cent synephrin, or 0.1 per cent p-hydroxyphenylpropanolamine solu-

\*From the Laboratory of Pharmacology, University of Illinois College of Medicine. Received for publication, January 15, 1934.

<sup>1</sup>An abstract of a thesis submitted in partial fulfillment for the degree of Doctor of Philosophy in Pharmacology in the Graduate School of the University of Illinois, 1934.

tion was exposed either in open Petri dishes (9.8 cm. diameter) or clear quartz covered dishes (6.8 cm. diameter) at a distance of 20 cm. from a Cooper-Howitt mercury arc lamp (35 ergs per mm.<sup>2</sup>) for from ½ minute to 20 hours at a temperature of 20° C. when water cooled or a temperature of 55° C. when air cooled.

*Boiling With H<sub>2</sub>O<sub>2</sub>.*—Ten cubic centimeters of the above solutions were boiled moderately in a 50 c.c. beaker over a free flame from 1 to 35 minutes with varying amounts of H<sub>2</sub>O<sub>2</sub> added.

*Irradiation With H<sub>2</sub>O<sub>2</sub>.*—Solutions were irradiated as in the first step, but with the addition of H<sub>2</sub>O<sub>2</sub> before exposure to the rays.

*Biologic Assay.*—Method 1: Change in blood pressure following intravenous injection in dogs was determined according to the method described in U.S.P. X, p. 212, for assay of liquor epinephrinae hydrochloridi. Anesthesia—nembutal (Abbott) 20 mg. per kg. intraperitoneally preceded by 3 mg. per kg. morphine sulphate subcutaneously; in a few cases—ether, preceded by morphine.

Method 2: The hyperglycemic and glycosuric actions following intraperitoneal injections of the drugs in rabbits were determined. Samples of blood and urine were taken from unanesthetized animals (starved 24 hr. or more) before the injection and 1 or 2 hours after injection of the drug. Blood sugar was determined by the Folin-Wu method and urinary sugar with Benedict's qualitative reagent.

Method 3: The inhibitory effect of the drugs on the isolated rat uterus using the usual means of recording contractions in aerated Locke-Ringer solution.

Method 4: The effect of intravenous injection of the drugs in relieving or preventing bronchial constriction induced by histamine in guinea pigs was studied according to the method of Koessler and Lewis.<sup>7</sup>

## RESULTS

*Epinephrine.*—Irradiation of a 1:10,000 solution of epinephrine HCl resulted in a loss of activity as shown in Table I with short intervals of exposure, and in Table II and Fig. 1, with long intervals. These results

TABLE I

Dog—Weight, 12 kg. Vagi atropinized  
Date—Sept. 13, 1932. Anesthesia—morphine, nembutal  
Injections: 0.2 c.c. epinephrine HCl 0.01 per cent  
Irradiation: 20 c.c. 0.01 per cent solution in quartz dish at 20 cm., air cooled; 2 c.c. samples removed at each time interval

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0.0	138	204	66	
2	0.5	136	192	56	
3	1.0	140	193	53	
4	1.5	150	196	46	
5	2.0	152	190	38	
6	3.0	150	182	32	
7	5.0	144	173	29	



confirm previous publications of our own and of other investigators<sup>1, 20, 6</sup> and fail to show any increase as reported by Ito and Terata. The difference in time necessary to produce these changes is due to the manner of irradiation.

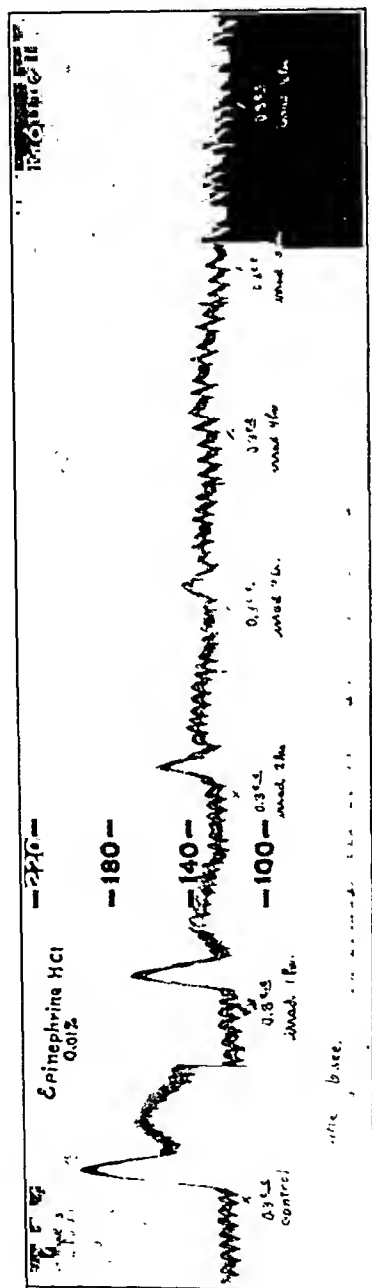


Fig. 1.

tion, the chief factors being distance from the arc, volume of solution, concentration of solution, and temperature. The predominance of depressor action in irradiated epinephrine solutions is seen in the 330-minute sample (Table II). This depressor action is eventually lost with continued irradiation.

tion. A 0.01 per cent solution of epinephrine alkaloid loses its activity by irradiation as does the HCl salt, the loss being even more rapid in the presence of  $\text{Na}_2\text{CO}_3$  (Table III). Irradiation of epinephrine HCl solution with the addition of  $\text{H}_2\text{O}_2$  markedly increases the rate of destruction (Table IV). Boiling the drug with  $\text{H}_2\text{O}_2$  also hastens its destruction (Table V).

TABLE II

Dog—Weight, 12 kg. Vagi atropinized  
 Date—Sept. 15, 1932. Anesthesia—morphine, nembutal  
 Injections: 0.3 c.c. epinephrine HCl 0.01 per cent  
 Irradiation: 20 c.c. 0.01 per cent solution in quartz dish at 20 cm., water cooled; 2 c.c. removed at each time interval

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	138	192	54	
2	60	136	168	32	
3	120	137	166	29	
4	180	141	156	15	
5	240	141	148	7	
6	300	144	148	4	
7	330	147	141	-6	
8	0	141	190	49	

TABLE III

Dog—Weight, 9 kg. Vagi atropinized  
 Date—June 16, 1931. Anesthesia—morphine, nembutal  
 Injections: 1 c.c. 0.01 per cent epinephrine alkaloid (P. D. and Co.) (see remarks)  
 Irradiation: 10 c.c. 0.01 per cent solution in Petri dish at 20 cm., air cooled, some with acid or alkali added (remarks)

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	48	145	97	
2	60	56	62	6	
3	60	60	69	9	Irradiated with HCl
4	60	62	63	1	Irradiated with $\text{Na}_2\text{CO}_3$

TABLE IV

Dog—Weight, 9 kg. Vagi atropinized  
 Date—July 19, 1933. Anesthesia—morphine, nembutal, ether  
 Injections: 0.2 c.c. of 0.01 per cent solution epinephrine HCl, except No. 5  
 Irradiation: 10 c.c. 0.01 per cent solution in quartz dishes at 20 cm., water cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	108	159	51	Control
2	35	110	149	39	No peroxide added
3	35	111	123	12	With 2 drops 2 per cent peroxide
4	35	109	111	2	With 5 drops 2 per cent peroxide
5	0	108	108	0	(Control peroxide—5 drops 2 per cent peroxide in 10 c.c. of water)

These same changes in epinephrine solutions are shown in tests of glycosuric action in rabbits (Tables VI and VII), on the relief of histamine bronchio-spasms in guinea pigs (Table VIII), and on the isolated rat uterine strip (Table IX).

TABLE V

Dog—Weight, 11 kg. Vagi cut  
 Date—Sept. 14, 1931. Anesthesia—morphine, nembutal  
 Injections: 0.5 c.c. epinephrine HCl 0.01 per cent  
 Oxidation: 10 c.c. 0.01 per cent solution boiled 10 minutes with 1 per cent  $H_2O_2$  (remarks)

NO.	MINUTES BOILED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	10	114	198	84	No peroxide
2	10	118	193	75	With 1 drop $H_2O_2$
3	10	113	172	59	With 2 drops $H_2O_2$
4	10	113	146	33	With 5 drops $H_2O_2$

TABLE VI

Rabbits—About 2.0 kg. wt.  
 Date—Aug. 14, 1931  
 Injection: Epinephrine HCl, 0.01 per cent, intraperitoneally  
 Irradiation: 30 c.c. samples 0.01 per cent solution in Petri dish at 20 cm., air cooled

RABBIT NO.	MINUTES IRRADIATED	DOSE MG.	BLOOD SUGAR			GLYCOSURIA
			NORMAL MG. PER 100 C.C.	AFTER INJECTION		
				HR.	MO. PER 100 C.C.	
1	0	1.0	121	3	235	++
2	60	1.5	129	3	125	-
3	60	3.0	118	3	115	-
4	0	1.0	167	1	290	++
5	60	3.0	118	1	267	+
6	180	10.0	139	1	180	-
7	0	0.5	110	1	258	++

TABLE VII

Rabbits—About 2 kg. in weight  
 Date—Sept. 6, 1931  
 Injections: Epinephrine HCl, 0.01 per cent, intraperitoneally  
 Oxidation: 20 c.c. 0.01 per cent solution boiled with 2 drops 1 per cent  $H_2O_2$

RABBIT	MINUTES BOILED	DOSE MG.	BLOOD SUGAR			GLYCOSURIA
			NORMAL MG. PER 100 C.C.	AFTER INJECTION		
				HR.	MG. PER 100 C.C.	
1	0	0.5	107	1	236	+
2	1	1.5	112	1	196	-
3	1	3.0	104	1	222	+
4	10	8.0	115	1	174	-

TABLE VIII

EFFECT OF EPINEPHRINE HCL ON RECOVERY FROM BRONCHIOLE CONSTRICTION INDUCED BY  
 0.06 MG. HISTAMINE PICRATE\*

Guinea pig—Weight 620 gm.  
 Date—Aug. 2, 1933. Anesthesia—pithed  
 Injections: Histamine picrate, 1 c.c. of 0.006 per cent solution intravenously. Epinephrine  
 HCl, 1 c.c. 0.005 per cent solution intravenously  
 Irradiation: 10 c.c. 0.01 per cent solution irradiated 90 minutes in quartz dish at 20 cm.,  
 air cooled

MINUTES AFTER HISTAMINE	0	1	2	3	4	5
Control	1	4	8	20	34	40
Irradiated epinephrine HCl at†	1	4	12†	23	32	39
Nonirradiated epinephrine HCl at†	1	3	8†	29	33	35

\*Figures represent excursion of lever in millimeters.

*Synephrin*.—Solutions of synephrin salts when exposed to ultraviolet rays regularly exhibit an increase in action as was shown in our preliminary report.<sup>1</sup> A maximum point is reached, however, when further irradiation effects a breakdown of the active substance until it is practically destroyed (Fig. 2 and Table X). The increased activity may be from 10 to 20 times the

TABLE IX

Epinephrine on uterine strip of rat

Date—July 27, 1933

Injections: Specified dose of 0.01 per cent epinephrine HCl added to cup containing uterine strip in 50 c.c. Locke-Ringer solution

Irradiation: 10 c.c. 0.01 per cent epinephrine HCl irradiated 90 minutes in quartz dish at 20 cm., air cooled

DOSE	DRUG	CONTRACTIONS (MOVEMENTS OF LEVER IN MM.)	
		NORMAL	AFTER INJECTION
0.01 c.c.	Epinephrine HCl nonirradiated	90	50
0.02 c.c.	Epinephrine HCl nonirradiated	90	-10
0.02 c.c.	Epinephrine HCl irradiated	90	42

TABLE X

Dog—Weight, 5.8 kg. Vagi cut

Date—Feb. 10, 1933. Anesthesia—morphine, ether

Injections: 1.0 c.c. synephrin, HCl 0.1 per cent

Irradiation: 20 c.c. 0.1 per cent solution in quartz dish at 20 cm., water cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. Hg)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	51	58	7	
2	360	53	114	61	
3	720	33	54	21	
4	1080	35	39	4	
5	1200	39	40	1	
6	0	42	50	8	

TABLE XI

Dog—Weight, 9 kg. Vagi atropinized

Date—Sept. 11, 1931. Anesthesia—morphine, nembutal

Injections: 0.5 c.c. synephrin, HCl 0.1 per cent

Oxidation: 10 c.c. 0.1 per cent solution irradiated with 2 drops 1 per cent  $H_2O_2$  in Petri dish at 20 cm., air cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. Hg)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	1	128	206	78	
2	4	130	270	140	
3	10	110	260	150	
4	20	102	256	154	
5	60	95	240	145	
6	120	100	233	133	

normal activity. Irradiation of the solution with the addition of a small amount of  $H_2O_2$  markedly decreases the time necessary to produce these changes (Table XI), and simply boiling the solution with  $H_2O_2$  effects a similar increase in activity (Table XII).

Irradiation of a solution of synephrin *base* for 60 minutes does not produce any increase in activity, while the addition of an acid makes the change

very evident (Tables XIII and XIV). The effect of varying the  $P_H$  of the synephrin solution is discussed later (under heading, "Chemistry"). These

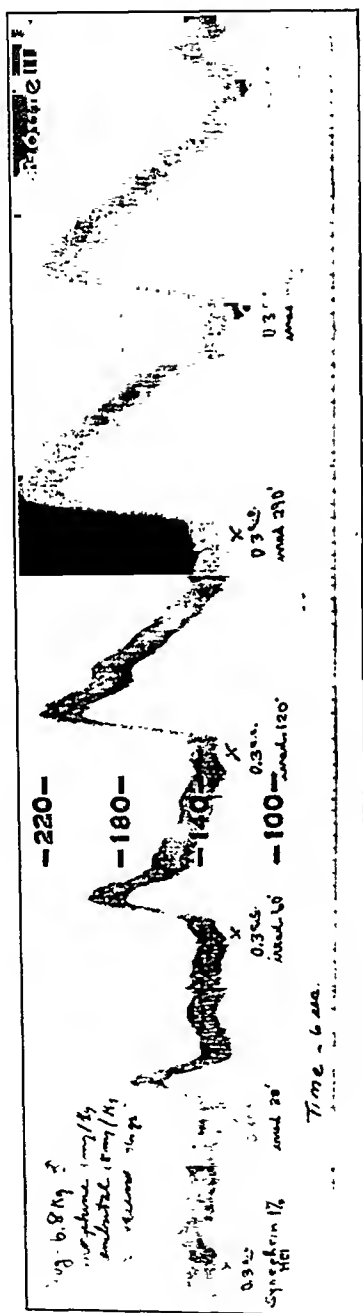


FIG. 2.

hyperactive solutions maintain their activity for a relatively long time since we have found that solutions kept in the laboratory for six months at room temperature were as active as when freshly prepared.

TABLE XII

Dog—Weight, 9 kg. Vagi atropinized  
 Date—Sept. 11, 1931. Anesthesia—morphine, nembutal  
 Injections: 0.5 c.c. synephrin HCl 0.1 per cent  
 Oxidation: 10 c.c. samples 0.1 per cent synephrin HCl (except No. 4) boiled with 2 drops of 1 per cent  $H_2O_2$

NO.	MINUTES BOILED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	100	110	10	1 per cent synephrin HCl boiled, then diluted to 0.1 per cent before injection
2	1	102	161	59	
3	4	104	187	83	
4	8	100	146	46	
5	10	99	226	127	
6	30	99	219	120	

TABLE XIII

Dog—Weight, 9 kg. Vagi atropinized  
 Date—Feb. 18, 1931. Anesthesia—morphine, ether  
 Injections: 0.5 c.c. of 0.1 per cent synephrin base or hydrochloride  
 Irradiation: 10 c.c. 0.1 per cent solution in Petri dish at 20 cm., air cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	150	159	9	} Synephrin base
2	60	152	161	9	
3	0	153	163	10	} Synephrin HCl
4	60	156	260	104	

TABLE XIV

Dog—Weight, 12 kg. Vagi atropinized  
 Date—Feb. 25, 1931. Anesthesia—morphine, nembutal, ether  
 Injections: 1.0 c.c. synephrin base 0.1 per cent (see remarks)  
 Irradiation: 10 c.c. of above solution in Petri dish at 20 cm., air cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	153	162	9	Irradiated with citric acid Irradiated with oxalic acid Irradiated with succinic acid
2	60	155	225	70	
3	60	154	221	67	
4	60	155	232	77	

TABLE XV

Rabbits—About 2.0 kg. in weight  
 Date—Aug. 24, 1931  
 Injections: Synephrin HCl 0.1 per cent intraperitoneally  
 Irradiation: 30 c.c. samples 0.1 per cent solution in Petri dish at 20 cm., air cooled

RABBIT	MINUTES IRRADIATED	DOSE MG.	BLOOD SUGAR			GLYCOSURIA
			NORMAL (MG. PER 100 C.C.)	AFTER INJECTION		
				HR.	MG. PER 100 C.C.	
1	0	40	122	1	111	-
2	60	35	105	1	115	-
3	180	35	139	1	303	++
4	180	20	119	1	250	++
5	180	30	93	2	200	++
6	0	100	120	2	138	-
7	0	300	89	2	129	-

The increased effectiveness of irradiated solutions of synephrin salts is demonstrated in the marked action of relatively small doses in producing hyperglycemia and glycosuria (Table XV). A marked hyperglycemia and glycosuria has been produced in rabbits with as little as 8 mg. of irradiated drug. Boiling the drug with  $H_2O_2$  increases this action also, as shown in Table XVI.

Irradiated synephrin solutions are effective in relieving histamine bronchospasms of guinea pigs (Table XVII) or preventing these spasms by previous injection of synephrin (Table XVIII). The same dose of nonirradiated drug had no noticeable effect.

TABLE XVI

Rabbits—About 2 kg. in weight

Date—Sept. 30, 1931

Injection: Synephrin, HCl 0.1 per cent, intraperitoneally

Oxidation: 20 c.c. samples of 0.1 per cent solution boiled with 2 drops 1 per cent  $H_2O_2$

RABBIT	MINUTES BOILED	DOSE MG.	BLOOD SUGAR			GLYCOSURIA PRODUCED
			NORMAL (MG. PER 100 C.C.)	AFTER INJECTION		
				HR.	MG. PER 100 C.C.	
1	0	100	111	1	142	—
2	1	40	109	1	121	—
3	5	40	117	1	186	—
4	10	20	114	1	232	++

TABLE XVII

Guinea pig—Weight, 533 gm.

Date—July 14, 1933. Anesthesia—pithed

Injections: Histamine (Pfanstiehl) 2 c.c., 0.001 per cent solution, intravenously. Irradiated synephrin HCl 2 c.c., 0.1 per cent, intravenously

Irradiation: 10 c.c. synephrin HCl (0.1 per cent) in quartz dish at 20 cm., air cooled, irradiated 90 minutes

	EXCURSION OF LEVER (MM.)		
	I CONTROL	II CONTROL	III WITH SYNEPHRIN
Normal excursion	60	70	60
0.02 mg. histamine injected	--	--	--
After 1 minute	1	1	1
After 2 minutes	1	2	1
After 3 minutes	2	3	20*
After 4 minutes	6	3	30

\*Injection of 2 mg. irradiated synephrin HCl here.

TABLE XVIII

RESPONSE OF BRONCHIOLES TO INJECTIONS OF 0.06 MG. HISTAMINE PICRATE AFTER PREVIOUS INJECTION OF SYNEPHRIN (EXCURSION OF LEVER—MM.)

Guinea pig—Weight, 620 gm.

Date—Aug. 2, 1933. Anesthesia—pithed

Injections: Histamine picrate (Nelson) 1 c.c. of 0.006 per cent solution, intravenously. Synephrin HCl, 1.2 c.c. of 0.1 per cent solution, intravenously

Irradiation: 10 c.c. 0.1 per cent synephrin HCl irradiated 90 minutes in quartz dish, air cooled, at 20 cm.

HISTAMINE	NO SYNEPHRIN		NONIRRADIATED SYNEPHRIN HCl		IRRADIATED SYNEPHRIN HCl	
	BEFORE	AFTER	BEFORE	AFTER	BEFORE	AFTER
After 7 min.	45	1	45	1	50	42
After 11 min.	35	1	35	1	50	14
After 15 min.	30	1	35	1	50	3
After 20 min.	32	1	36	1	50	1

The isolated rat uterine strip shows the increased effectiveness of irradiated synephrin in inhibiting normal contractions (Table XIX).

*Parahydroxyphenylpropanolamine.*—This drug reacts to ultraviolet irradiation with an increased pressor action. More than ten times the activity of the nonirradiated drug is shown in Table XX.

TABLE XIX

Synephrin on uterine strip of rat

Date—Sept. 27, 1933

Injections: Specified dose of 0.1 per cent synephrin HCl added to cup containing uterine strip in 50 c.c. Locke-Ringer solution

Irradiation: 10 c.c. 0.1 per cent synephrin HCl irradiated 90 minutes in quartz dish at 20 cm., air cooled

*Uterine Strip No. 1*

DOSE	DRUG	CONTRACTIONS (MOVEMENT OF LEVER IN MM.)	
		NORMAL	AFTER INJECTION
0.2 c.c.	Synephrin HCl nonirradiated	+96	+82
0.2 c.c.	Synephrin HCl irradiated	+98	-10
1.0 c.c.	Synephrin HCl nonirradiated	+96	- 6

*Uterine Strip No. 2*

0.1 c.c.	Synephrin HCl nonirradiated	75	50
0.1 c.c.	Synephrin HCl irradiated	80	-25

TABLE XX

Dog—Weight, 15.4 kg. Vagi atropinized

Date—Apr. 9, 1933. Anesthesia—morphine, nembutal

Injections: 0.5 c.c. of p-hydroxyphenyl-1-amino-2-propanol-1 hydrochloride. Control solution 0.1 per cent—irradiated solutions 0.01 per cent

Irradiation: 20 c.c. 0.01 per cent solution in quartz dish at 20 cm., air cooled

NO.	MINUTES IRRADIATED	BLOOD PRESSURE (MM. HG)			REMARKS
		NORMAL	AFTER INJECTION	DIFF.	
1	0	116	140	24	Control 0.1 per cent 0.01 per cent solutions
2	15	118	152	34	
3	35	116	153	37	
4	60	114	142	28	
5	125	114	128	14	

#### OXIDATION PRODUCTS OF EPINEPHRINE

We found that heating the drug with hydrogen peroxide and treatment with ultraviolet light, effects similar pharmacodynamic changes in the drug. For this reason and because of the relative simplicity in using hydrogen peroxide, this method was chosen in preparing the drugs for the study of the oxidation products.

*Procedure:* Five-tenths gram of pure epinephrine base was oxidized by refluxing with 100 c.c. of neutralized 5 per cent  $H_2O_2$  for fifteen minutes. The solution developed a reddish brown color.

*Tests for Oxidation Products.*—1. Unchanged Epinephrine: The solution was cooled at once in ice water and excess oxygen removed by evacuation (in a flask at 1/150 atmosphere). The solution was first made acid with  $H_2SO_4$  and distilled, then made alkaline with NaOH and distilled. The residue in the distilling flask when treated with phosphomolybdotungstic acid gave



an amethyst color, indicating the presence of epinephrine or catechol, both of which give this color with this reagent.

When  $\text{FeCl}_3$  solution was added to the residue, a greenish blue coloration changing to red was produced, again indicating the presence of epinephrine or catechol.

Mitchell's reagent (0.2 per cent  $\text{FeSO}_4$  + 1 per cent  $\text{KNaC}_4\text{H}_4\text{O}_6$ ) gives with epinephrine a purple color and with catechol a pale blue. When this reagent was added to the residue, a pale blue color, changing to a purple, was observed.

$\text{NH}_4\text{OH}$  with epinephrine produces a yellow color changing to orange and to pink; with catechol, it gives a green color, which fades rapidly. This reagent with the residue gave first a green color changing to orange and to pink and faded out.  $\text{NH}_4\text{OH}$  with Mitchell's reagent gives for both compounds a reddish violet changing to red. The same colors were obtained with the residue.

These tests indicate that the oxidized material still contained some unchanged epinephrine. This fact was further proved by blood pressure tests on a dog.

2. Catechol: Tests on the residue indicated the presence of catechol. The acid distillate when tested with phosphomolybdic acid reagent gave a violet color; with ferric chloride solution a greenish blue; and with Mitchell's reagent a pale blue. Since epinephrine would not distill over, these tests confirm the presence of catechol. Millon's reagent with the distillate gave a negative test for the phenol group.

3. Ethyl Alcohol: When 5 c.c. of the acid distillate and 5 c.c.  $\text{H}_2\text{SO}_4$  were titrated with  $\text{K}_2\text{Cr}_2\text{O}_7$  (19 gm./liter), about 2 c.c. was required (Nieloux method). During the oxidation, the odor of acetaldehyde was perceptible, indicating the presence of an alcohol. When a few cubic centimeters of the distillate was warmed with a few drops of benzoyl chloride and  $\text{NaOH}$ , the odor of ethyl benzoate was observed, thus proving the presence of ethyl alcohol.

4. Methyl Alcohol: This test was negative by the U.S.P. (X) method.

5. Acetic Acid: The distillate was acid to litmus and, when heated, had the characteristic odor of acetic acid. When treated with a small amount of valeric acid and  $\text{FeCl}_3$ , it gave a reddish violet color, indicating acetic acid.

6. Formic Acid: No formic acid was found.

7. Aldehyde: No test for aldehyde was obtained on the above distillate, probably because the oxidation was carried too far. To check this, a 0.1 per cent solution of epinephrine base was oxidized in the cold for a few minutes with  $\text{H}_2\text{O}_2$ , evacuated a few minutes, and then distilled. The first few cubic centimeters of distillate gave a positive reaction with Schiff's reagent (fuchsin sulphurous acid) and only a yellow color with morphine and sulphuric acid, thus proving the absence of formaldehyde and the presence of acetaldehyde.

8. Methylamine: The alkaline distillate had an ammoniacal fishlike odor, characteristic of methylamine. Rimini's test (1 c.c. acetone and 1 drop dilute

sodium nitroprusside) for an aliphatic amine was strongly positive, thus indicating the presence of methylamine.

From a study of these oxidation products, it appears that epinephrine, when oxidized, splits off the side chain, leaving the catechol nucleus. From the side chain, we first obtain ethyl alcohol and methylamine. Further oxidation of the alcohol yields acetaldehyde which in turn is oxidized to acetic acid. Catechol is the only decomposition product which has any appreciable effect on blood pressure and then very weak as compared to epinephrine.

The identification of these oxidation products is apparently sufficient evidence why epinephrine loses its pressor action when oxidized.

#### OXIDIZED SYNEPHRIN (CHEMICAL)

Five cubic centimeters of 10 per cent synephrin hydrochloride was boiled on a hot plate with 1 drop of 30 per cent superoxol until a deep red color developed—indicating the point when approximately maximum activity is reached.

Since synephrin base precipitates with  $\text{NH}_4\text{OH}$  in a 10 per cent solution, it was thought possible that the oxidized product might be separated in the same manner. Accordingly, the oxidized solution was cooled and 4 drops of concentrated  $\text{NH}_4\text{OH}$  added. The resulting precipitate was filtered on a suction filter, washed, and dried in a vacuum desiccator over  $\text{H}_2\text{SO}_4$ .

##### A. Precipitate tests:

1. Melting point of precipitate— $170$  to  $171^\circ \text{C}$ .  
Melting point of original synephrin base— $173^\circ \text{C}$ .
2. On blood pressure of dog:  
A 0.1 per cent solution of the precipitate had the same effect on blood pressure as the same concentration of synephrin base.
3. Chemical tests:
  - a. Solution of the precipitate had a pale pink brown color.
  - b. Test with Mitchell's reagent ( $\text{FeSO}_4$  + Rochelle salt) shows no epinephrine or catechol.
  - c. With  $\text{FeCl}_3$ —no catechol.
  - d. With phosphomolybdotungstic acid—same color as with synephrin base.
  - e. With phosphotungstic acid reagent +  $\text{Na}_2\text{CO}_3$  (N.N.R.)—blue color a little darker than same amount of synephrin base—but not nearly as dark as epinephrine.

##### B. Test on filtrate:

On blood pressure of dog: Very slight activity which was not more than one-tenth of the activity of the original oxidized solution, but approximately the same as that produced by the same dose of synephrin base.

From the results of these tests, it appears that the precipitate obtained by alkalization of the oxidized synephrin solution is only the unchanged

synephrin base. Furthermore, since the filtrate was not hyperactive, the active material seems to have been lost when the solution was alkalinized with  $\text{NH}_4\text{OH}$ . Combining the precipitate and filtrate and injecting into a dog gave no more rise in blood pressure than a simple additive effect of the two solutions.

These tests show only that the hyperactive oxidation product of synephrin is not precipitated by  $\text{NH}_4\text{OH}$  and is apparently rendered inactive by this reagent.

#### THE EFFECT OF HYDROGEN ION CONCENTRATION IN IRRADIATION OF SYNEPHRIN BASE

In earlier experiments, it was found that irradiation of synephrin base did not change its pressor activity whereas the hydrochloride, tartrate, citrate, oxalate, or succinate gave a marked change. As a further study of the effects of ultraviolet light on this drug, samples of 0.1 per cent synephrin base were made up and adjusted to a range of  $P_H$  values by the addition of N/20 HCl. A La Motte colorimetric outfit was used to determine the  $P_H$  values, all buffer solutions being freshly prepared. The value of the 0.1 per cent synephrin base solution was found to be  $P_H$  9.3; N/20 HCl was added as indicated in Table XXI to make up solutions of  $P_H$  9.0, 8.0, 7.0, 6.0, 5.0, 4.0, and 3.0.

TABLE XXI

		c.c. N/20 HCl	$P_H$
15 c.c. 0.1 per cent synephrin base	+	0	9.3
15 c.c. 0.1 per cent synephrin base	+	0.3	9.0
15 c.c. 0.1 per cent synephrin base	+	1.3	8.0
15 c.c. 0.1 per cent synephrin base	+	1.65	7.0
15 c.c. 0.1 per cent synephrin base	+	1.73	6.0
15 c.c. 0.1 per cent synephrin base	+	1.77	5.0
15 c.c. 0.1 per cent synephrin base	+	1.81	4.0
15 c.c. 0.1 per cent synephrin base	+	2.15	3.0

Ten cubic centimeters of each sample were irradiated for ninety minutes in quartz-covered, water-cooled dishes, and when tested for pressor action in a dog, showed the following:

1. Synephrin base,  $P_H$  9.0 or above, develops a reddish brown color after irradiation; its action is not increased but is somewhat decreased.
2. The solutions of  $P_H$  7.0 and lower are markedly increased in activity by ultraviolet irradiation. The color developed was a paler brown color than the  $P_H$  9.0.
3. Solution of  $P_H$  8.0 had an increased action but not as much as those of lower  $P_H$ .
4. Solutions of the whole  $P_H$  range had the same activity before irradiation.

This experiment shows that in order to develop the increased activity, synephrin must have an acid present. The amount of acid necessary to produce maximal effect is approximately the amount needed to form just completely the acid salt of the base present. It is left then to determine what rôle the acid plays in bringing about the formation of the active ingredient produced when synephrin is irradiated.

5. All samples become more acid when irradiated. Heating the irradiated solution with alcohol and concentrate  $H_2SO_4$  gives the odor of ethyl acetate indicating the presence of acetic acid. The Table XXII shows the  $P_{II}$  before and after ninety minutes' irradiation and pressor activity of the irradiated drug. The change in  $P_{II}$  may be due to the acetic acid which is formed as an oxidation product of the irradiation.

TABLE XXII

$P_{II}$		ACTIVITY OF IRRADIATED DRUG ON DOG BLOOD PRESSURE (MM. HG.)		
BEFORE IRRADIATION	AFTER IRRADIATION	NORMAL	AFTER INJECTION	DIFF.
9.0	6.5	128	140	12
8.0	3.6	127	194	67
7.0	3.0	114	234	120
6.0	2.9	108	176	68
5.0	2.9	118	166	48
4.0	2.8	116	156	40
3.0	2.5	118	156	38

6. Solution of synephrin base when irradiated for seven hours had a slightly increased action on blood pressure and showed a  $P_{II}$  of 4.7.

#### DISCUSSION

##### *Epinephrine*

The literature on the effect of ultraviolet rays on epinephrine solutions is not very extensive. If we consider that ordinary daylight provides some ultraviolet rays originating from the sun, we may have a possible explanation of the changes taking place in dilute solutions of epinephrine salts commonly observed. Such dilute solutions are stated (N.N.R. 1933—p. 194) to rapidly lose their strength, the deterioration being accompanied by a reddish or brownish discoloration. For this reason, solutions of epinephrine are usually discarded as soon as any color develops although they may still be practically as active as when freshly prepared. There is considerable hesitancy about sterilizing an epinephrine solution by boiling for this same reason. It is possible that our opinion of the rapidity of destruction of epinephrine solution is somewhat exaggerated. Richter and Gerhartz<sup>13</sup> in 1908, while studying the action of roentgen rays on ferments, made a brief study of the action of soft and hard roentgen rays on solutions of epinephrine. By testing on the blood pressure of dogs, they found some evidence of destruction of pressor action, but there was too much variation to draw definite conclusions. Ludin<sup>8</sup> in 1918 stated that roentgen rays did not alter adrenalin action as judged by the action on the surviving intestine of rabbits. McGuigan and Mostrom<sup>10</sup> in 1913 reported the loss of glycosuric action of epinephrine boiled with sodium hydroxide, while some pressor action still remained. This work supported that of Ransom<sup>12</sup> in 1911. Savopol in 1914 exposed solutions of 1:1,000 adrenalin to the rays from a quartz mercury arc lamp and reported changes in some minor actions of adrenalin. 1. The hemoagglutinating and hemolytic properties were not diminished even with three hours' irradiation but rather increased to a certain extent. 2. The power of adrenalin to neu-

tralize tetanus toxin was lost by three hours of irradiation. 3. The necrotizing property of adrenalin on subcutaneous injection was attenuated with ten minutes' irradiation and entirely lost with one and one-half hours of irradiation. The effect of ultraviolet irradiation on the pressor action of epinephrine was reported by Verda, Kneer, and Burge in 1931. This method of judging the strength of epinephrine solutions is recognized by the U.S.P. X. After thirty-five minutes of irradiation, the pressor action was practically destroyed, and instead there appeared considerable depressor action. Ito and Terata also in 1931 reported an increase in the action of epinephrine solutions after irradiation for a short time and a loss of action by longer periods of irradiation. Another independent investigation by Ewing, Blickensdorfer, and McGuigan in 1931<sup>10</sup> on epinephrine and related products showed a loss of pressor action of epinephrine solutions, which was almost complete in samples irradiated ninety minutes. Kneer, Orth, Verda, and Burge in 1931 investigated the effect of ultraviolet irradiation and reported the loss of depressor action which nonirradiated solutions give when very dilute.

#### SYNEPHRIN

Since synephrin is a rather new drug, reports on its action are not very numerous. Tainter and Seidenfeld in 1930<sup>10</sup> made a quite thorough study of sympathomimetic compounds, including synephrin. They reported the stability of solutions of the drug to boiling, and studied its sympathomimetic action on blood pressure, blood vessels, intestine, bronchioles, and the property of chemosis. In 1931, Ewing, Blickensdorfer, and McGuigan first reported a marked increase in pressor action of synephrin salt solutions after irradiation for ninety minutes. Higgins, Ewing, and McGuigan in 1932<sup>4</sup> reported the effect of irradiated synephrin solutions on the dog heart—which effect could not be obtained with the nonirradiated drug. Stockton, Paee and Tainter in 1931<sup>18</sup> reported the clinical value of racemic synephrin in raising blood pressure, relieving asthmatic attacks, as an adjuvant to procaine in local anesthesia, and for shrinkage of mucous membranes with topical application.

#### OTHER DRUGS

Investigations of the action of ultraviolet light on drugs was stimulated by a rather extensive study of the formation of vitamin D. Rosenheim and Webster in 1928<sup>14</sup> showed that ergosterol alone, when treated photochemically (by ultraviolet light) produced vitamin D, and concluded therefore that ergosterol was the parent substance of this vitamin.

Pacini and McGuigan in 1930 reported the detoxification of 0.1 per cent solutions of nicotine when exposed to ultraviolet light. In 1931, Higgins, Ewing, and McGuigan showed that nicotine solutions were weakened by simple evaporation, but that when evaporation was prevented, ultraviolet rays still caused a destruction of nicotine. Wakeham and Traey<sup>21</sup> in 1932 reported the effect of ultraviolet irradiation on 100 per cent and 10 per cent nicotine solutions. They reported a maximum destruction of toxicity in solutions irradiated one and one-fourth hours. Irradiation for a longer time resulted in an increase in toxicity. Gant<sup>2</sup> has recently published a paper on

the irradiation of nicotine showing that decomposition only takes place and that the decomposition products are finally nitrates, nitrites, and ammonia. Macht in 1932 reported the action of ultraviolet light on solutions of digitalis. He found a decrease in toxicity up to one hour and thirty minutes of irradiation, then an increase in toxicity up to two hours and fifteen minutes of irradiation followed by subsequent decrease.

Many of the conflicting or varying results of different investigators can be explained by the different methods used, either in irradiating or in methods of judging the activity of the drug. The first factor is the source and intensity of light used. This is usually stated in such investigations so that any one can readily evaluate this factor. The distance of the solution from the source of light is of equal importance in judging how much energy is actually reaching the substance irradiated. The volume of solution and surface area exposed to the rays has a great influence on the effect of the irradiation since the rays penetrate only slightly beneath the surface of the solution. Another factor of great importance is the concentration of the solution irradiated. The destruction of nicotine solutions reported by Pacini and McGuigan in 1930 was based on the irradiation of 10 c.c. of a 0.1 per cent solution in a Petri dish having an area of approximately 75 sq. cm. exposed to the rays. This means that 10 mgm. of nicotine were destroyed after ninety minutes of irradiation at 12 inches from the source. Wakeham and Tracy in their work on nicotine reported in 1932 irradiated 100 per cent and 10 per cent solutions in quartz tubes, the volume and surface area exposed not being stated. Their method of judging destruction of nicotine was by determining the M.L.D. in white rats. We point out these investigations as examples of differences in methods used and the inadequacy of some of these methods, which factor is probably the reason for the different results obtained. We have found that a 0.1 per cent solution of synephrin hydrochloride solution is markedly changed by ultraviolet irradiation whereas a 1.0 per cent solution irradiated under the same conditions is only slightly changed. Likewise epinephrine hydrochloride solutions in a 1:10,000 dilution are quite rapidly destroyed by the ultraviolet rays; whereas in a 1:1,000 dilution, very little change occurs under similar conditions. We have noted that the temperature of the irradiated solution also affects the results. At a higher temperature changes take place more rapidly presumably because these changes are usually photochemical reactions which are hastened by increased temperature.

In our work, we have confirmed previous investigations showing that ultraviolet light destroys the activity of epinephrine solutions as judged by various methods, and in no instance have we found any evidence of potentiation of this drug. Heating the solution of epinephrine hydrochloride with  $H_2O_2$  produced similar changes, both chemically and in biologic activity. The chemical products resulting from oxidation explain the loss in activity.

The increased biologic activity of synephrin solutions effected by ultraviolet irradiation has been shown by various methods used in this investigation. Similar changes effected by heating with  $H_2O_2$  lead us to believe that the

changes are only the result of oxidation. The exact nature of the hyperactive substance formed when solutions of synephrin salts are irradiated is difficult to ascertain, since a combination of unchanged synephrin with several oxidation products is present in the hyperactive solution. We hope in the near future to succeed in identifying this substance. The necessity of the presence of some acid with the synephrin base in developing the hyperactive substance is established.

The brief study of the hydrochloride of parahydroxyphenylpropanolamine gives evidence of changes similar to those with synephrin hydrochloride and indicates that the important change is probably in the phenyl group rather than in the side chain.

### CONCLUSIONS

1. Epinephrine solutions are oxidized by ultraviolet light so that all normal actions of the drug are completely lost.
2. No evidence has been found to indicate any activation of epinephrine solutions by ultraviolet irradiation.
3. Some decomposition products of irradiated epinephrine have been identified.
4. Irradiation of solutions of synephrin salts increases their action many times as judged by all methods of standardization used.
5. Solutions of synephrin base do not exhibit any appreciable increase in action following irradiation.
6. Excess irradiation of hyperactive solutions of synephrin salts destroys their activity.
7. Irradiation of parahydroxyphenylpropanolamine increases its action approximately to the same extent as is found with synephrin salts.

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## THE RÔLE OF AMIDOPYRINE IN THE ETIOLOGY OF GRANULOCYTOPENIA WITH SPECIAL REFERENCE TO ITS CHEMICAL STRUCTURE

LUCIUS FELIX HERZ, PH.B., M.D., NEW YORK, N. Y.

GRANULOCYTOPENIA (otherwise known as agranulocytosis, agranulocytic angina, or granulopenia) was first accurately described by Schultz<sup>1</sup> in 1922. Beckman<sup>2</sup> mentions the fact that the end-results of the disease were demonstrated by Pepper as long ago as 1857, but the blood picture was not recognized until Schultz's researches.

Beckman<sup>2</sup> gives an excellent description of the disease. He states that it is first characterized by a complete elimination of the myelocytes, followed in four days by immense reduction in the granular leucocytes, and later their complete absence. Two days later there are extreme symptoms of sudden collapse, chill, fever, injected pharynx or ulcerative stomatitis, sometimes jaundice, soon stupor or death (unless the maturation of myelocytes is resumed). General sepsis develops if the patient survives this stage, and death soon follows.

Benzene and the benzene ring are sometimes blamed for the causation of the disease. Kracke produced the disease in rabbits by injecting benzene into the circulation.<sup>3</sup> As benzene is rarely used as such in medicine, and then chiefly in the treatment of leucemia, and as the compounds bearing the benzene ring which we use therapeutically are well tolerated when given in proper doses (see the author's article on acetanilid<sup>11</sup>), I will show later why amidopyrine must be considered in a different category.

According to Madison and Squier,<sup>4</sup> 500 cases of granulocytopenia are now on record, the increase in the number of cases on record closely paralleling the increase in the use of amidopyrine within recent years. The authors ascribe the condition in 14 of their cases to "benzene ring derivatives," and later admit that they referred to amidopyrine. I am prepared to show that amidopyrine should not be classed as a benzene ring derivative.



Madison and Squier<sup>5</sup> in a later article give a detailed description of 14 cases of granulocytopenia, all of which followed the use of amidopyrine (7 with barbiturates, 6 alone, and 1 combined with other drugs). Eight patients died. The surprising part was that physiologic doses were used in all cases (one patient took amidopyrine gr. x daily for eleven days and developed the disease). The authors conclude their article as follows:

"(1) The increase in incidence of primary granulocytopenia (agranulocytic angina) has paralleled the increase in the use of drugs containing amidopyrine, and especially those containing amidopyrine with a barbiturate.

"(2) The disease has appeared most frequently in persons apt to be taking drugs: *physicians, nurses, and those directly under the care of a physician.*

"(3) In each of 14 patients the onset of primary granulocytopenia was directly preceded by the use of amidopyrine alone or in combination with a barbiturate.

"(4) The mortality in a group of 6 patients who continued the use of drugs containing amidopyrine was 100 per cent. In a group of 8 patients who did not continue the use of these drugs, only two died, and both of these died in the initial attack.

"(5) The administration of a single dose of amidopyrine to each of two patients who had recovered from the acute disease was followed by a rapid profound fall in the granulocytes.

"(6) One rabbit was given allylisopropylbarbituric acid with amidopyrine (allonal) by mouth in relatively large doses, showed an abrupt drop in granulocytes and died on the thirteenth day. Preceding death, there was complete absence of granulocytes in the peripheral blood; 17 other rabbits given allonal or amidopyrine showed no significant changes in the blood picture.

"(7) We believe that amidopyrine alone or in combination with a barbiturate is capable of producing primary granulocytopenia in certain individuals who have developed sensitivity to the drug.

"(8) We believe that the appearance of primary granulocytopenia following the use of such drugs may be the result of an allergic or anaphylactoid drug reaction."

Armand J. Quick, in a recent article,<sup>6</sup> and also in a letter to the *Journal of the American Medical Association* states that drugs which produce allergy may perhaps cause a severe inflammatory reaction which may terminate in neurosis.

Hare<sup>8</sup> has collected 121 cases of untoward effects from antipyrine, most of which had erythematous patches and bullae. The dose was usually a moderate one. Amidopyrine is derived from antipyrine and is structurally similar.

Randall<sup>9</sup> describes a case of granulocytopenia following the use of barbiturates and amidopyrine. He blames the "benzene ring" in both drugs. I cannot agree with his conclusions as I will state in more detail later.

Zinniger<sup>10</sup> reports two cases of granulocytopenia of unknown origin and in a letter to the editor sent later, ascribes them to amytal compound (containing amidopyrine).

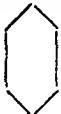
Hoffman, Butt, and Hickey<sup>12</sup> presented a series of 14 cases of granulocytopenia with 13 deaths. The one patient who recovered had developed the disease following dinitrophenol. The 13 who died developed the disease following the use of amidopyrine.

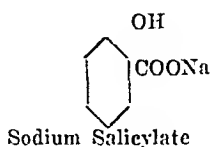
In November, 1933, Watkins<sup>13</sup> presented 32 cases of granulocytopenia which were observed at the Mayo Clinic. Of these 24 had taken amidopyrine or a barbiturate. Ten took amidopyrine alone and of these 2 died. One of the 8 who recovered had 12 attacks within two and a half years. He took amidopyrine for the relief of migraine and developed granulocytopenia after each session. One attack followed the use of ethyl methyl butyl barbiturate. Two patients used allonal, neither died; 5 used amytal, all died; 4 took sodium ethyl methyl butyl barbiturate, all died in the first attack. Two took phenobarbital, one died. Eight patients showed no definite history of drug taking, but had been under the care of physicians for chronic ailments, and it is thought that they had been taking either amidopyrine or the barbiturates. Watkins states: "Whether these persons have primary deficiency of the bone marrow is open to question, but before the onset of the neutropenic state, the leucocyte response in most instances had been normal. The fact that in two cases recurring neutropenia has regularly followed the use of amidopyrine, would indicate that in these cases there is apparent association between ingestion of the drug and neutropenia. The possible relationship between these drugs and granulocytopenia is not, in my opinion, a general contraindication to their use, but when they are to be used regularly, it would seem advisable to make a leucocyte count at intervals of every two or three days while the patient is in a hospital and every two or three weeks if the use of the drug is continued after the patient's dismissal from the hospital."

Hoffman, Butt, and Hickey<sup>12</sup> state also that they are conducting a series of experiments with rabbits. They state "amidopyrine alone was used in contrast to the combination of Madison and Squier of amidopyrine with allyl-isopropyl barbituric acid, because *amidopyrine was the only drug used in 13 of the 14 cases observed.*" They succeeded in producing a marked leucopenia in the rabbits ranging from 8 per cent to 20 per cent of normal.

The authors again blame the benzene ring for the leucopenia, but state "whether the latter (the benzene ring) is the actual toxic agent in the production of neutropenia needs further experimentation, part of which we are now undertaking. Our work points to amidopyrine as having a definite effect on myeloblastic tissue similar in man and rabbits. Whether this is an individual susceptibility of the nature of an allergic reaction as suggested by Pepper,<sup>14</sup> remains to be determined. *Until it is, the use certainly of amidopyrine alone or in combination with other drugs should be restricted to patients having leucocyte counts several times a week.*"

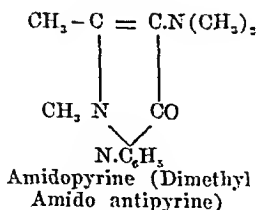
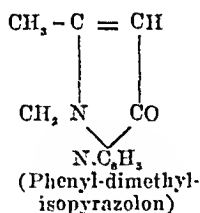
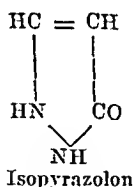
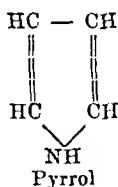
Beckman<sup>2</sup> is one of the few writers who seem to have correctly classified amidopyrine according to its structural formula, not as a benzene ring derivative, but as a *pyrazolon derivative*. It is true that there is a phenyl radicle present, but as this is a side chain of the pyrazolon nucleus, the pyrazolon is the active element in the drug.

Let us see the chemical nature of some of the antipyretics in common use. First we will consider derivatives of the benzene ring,  or  $C_6H_6$ .



All these compounds except benzene (which is not given therapeutically except in rare instances) are relatively nontoxic when given in therapeutic doses. Even in immense overdoses no cases of granulocytopenia have been traced to them. It is thought that acetanilid and phenacetin are gradually converted to para-amino-phenol which exerts an antipyretic action, and then excreted as the glycuronate or sulphate of the above compound.<sup>15</sup> Salicylates are excreted either as such or as modified glycuronates.

Amidopyrine and antipyrine belong to a different group. Instead of the hexagonal benzene ring, they are built about the pyrazolon ring, a modification of pyrrol, both being pentagons:

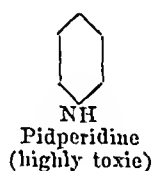
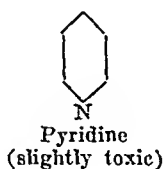
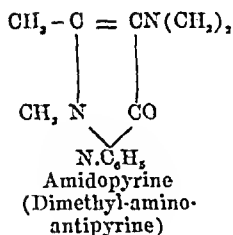
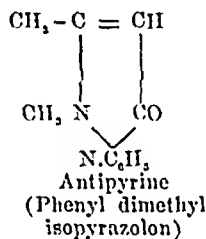
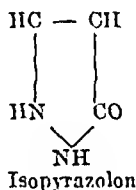
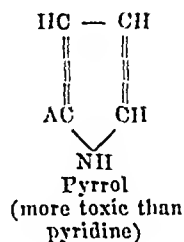
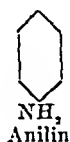


Why is amidopyrine more toxic than acetanilid, phenacetin or aspirin? I shall endeavor to answer that question from an analysis of its chemical structure. The fact that the pentagonal pyrrol ring instead of the hexagonal benzene ring is present is not the essential point, in my estimation. I consider the NH component of the ring as the toxic element, regardless of whether it remains as NH or whether the H is replaced by another radicle such as phenyl as in antipyrine or amidopyrine.

McGuigan<sup>15</sup> states "although antipyrine is derived from phenyl hydrazine, which is a strong blood poison, it is physiologically not a phenyl hydrazine

derivative, perhaps on account of the formation of the pyrazolon ring." McGnigan started with the correct iden, but as the toxicity of amidopyrine had not been fully appreciated, his final conclusion is not correct. McGnigan hit the nail on the head when he mentioned that antipyrine is derived from phenyl hydrazine. I maintain that it not only is derived from phenyl hydrazine, but partakes of its nature.

I will show several drugs having this NH or N radicle as an integral part of a ring:



Pyridine is slightly toxic. Piperidine is highly toxic and differs from pyridine solely in the substitution of an NH (imido) radicle for the N radicle. This shows the toxic character of the imide radicle. Furthermore, all alkaloïds, such as morphine, nicotine, quinine, strychnine, cocaine, etc., contain this toxic radicle.

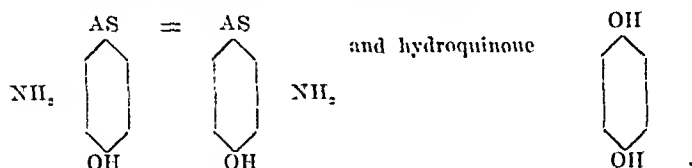
Amidopyrine and antipyrine have this imido radicle with the H replaced by a benzene ring, which apparently is insufficient to neutralize the toxicity of the imido radicle.

Phenyl hydrazine contains the imido radicle, not as an integral part of the ring, but as a closely joined side chain.

Examining other chemical combinations, we find the NH<sub>2</sub> (amino) radicle as a component of proteins, amino acids, and such end-products as urea and uric acid. It is nontoxic in most of its combinations, but a few compounds such as anilin, when not combined with side chains are toxic. Side chains decrease the toxicity to a marked extent, and permit of the use of accepted benzene derivatives with complete safety in medicine.

Barbituric acid and the barbiturates are products synthesized from urea and malonic acid, and present an incomplete purine ring—not a benzene ring. Uric acid may be synthesized in the laboratory from barbituric acid.

The writer does not maintain that amidopyrine is the sole cause, or that the imido radicle is solely responsible for the drug causation of granulocytopenia. He merely states that it is one of the important causes. Other drugs may likewise cause granulocytopenia, and the blame must be placed upon other equally toxic radicles. For instance dinitrophenol consists of a benzene ring with one (OH) and two (NO<sub>2</sub>) radicles. The NO<sub>2</sub> radicles are quite as toxic as the NH. Picric acid is trinitrophenol, and is highly toxic. Other drugs which have caused granulocytopenia are salvarsan:



and likewise some of the barbiturates are said to have caused it.

Having demonstrated the toxic nature of amidopyrine, and having shown that it has produced more cases of granulocytopenia than any other drug in common use in medicine, the thinking members of the medical profession should consider amidopyrine and its progenitor antipyrine as too dangerous for use in medicine. Why should we use a drug with fear and trepidation, and be compelled to check its use with a leucocyte count every two days, as Watkins suggests, when we have safe and efficient drugs to answer our therapeutic requirements? There is no reason, in the light of present scientific investigation, ever to use the dangerous pyrazolon compounds, amidopyrine and antipyrine.

The writer<sup>11</sup> has shown that acetanilid is a safe and efficient drug when properly used. He has shown the unscientific nature of most of the adverse propaganda. Even the idea that caffeine should not be combined with acetanilid, as it was thought to form a toxic combination, has been exploded by McGuigan who showed by animal experimentation that caffeine decreases the toxicity of acetanilid. Other investigators have proved that citrates still further diminish its toxicity. A combination of acetanilid, caffeine, and potassium citrate would form an excellent and relatively nontoxic combination.

Lowy, and Helms, in a paper which I have had the privilege of reading, and which has not yet been published, based upon a questionnaire sent by Dr. Lowy to every hospital in the United States asking about the number of deaths, poisoning, and addiction from opiates, barbiturates, and amidopyrine, antipyrine, acetanilid, and phenacetin during a ten-year period shows the presence of far more cases of deaths and addictions from the barbiturates than from the antipyretics. There were practically no deaths from the latter group, but as granulocytopenia was then not generally recognized, and complete blood counts were not done in all cases, many deaths from unrecognized causes may

have been from granulocytopenia. Acetanilid, in spite of all the adverse propaganda against it, was rarely considered the cause of death, practically never as an addiction, and rarely as a cause of toxic phenomena.

Acetphenetidin is similar in its action to acetanilid but less efficient, and consequently requires large doses. In doses sufficient to produce results, it is more toxic than acetanilid.

### CONCLUSIONS

1. Granulocytopenia has increased markedly in prevalence within the past few years. It is a virulent and rapidly fatal disease, and it has been proved beyond any reasonable doubt that amidopyrine is the most frequent cause of this condition.

2. Madison and Squier<sup>5</sup> have produced granulocytopenia in one dog by a combination of amidopyrine and allylisopropyl barbituric acid (allonal), while Hoffman, Butt, and Hickey<sup>12</sup> have produced granulocytopenia in several rabbits by feeding amidopyrine alone.

3. Amidopyrine is a pyrazolon compound containing the highly toxic NH (imido) radicle, is derived from phenyl hydrazine, and resembles the latter in its rapid reduction of granular leucocytes in the blood stream and bone marrow.

4. Fully 500 cases of granulocytopenia have been described within recent years, and the increase in its prevalence closely parallels the increase in the use of amidopyrine, the majority of patients having used it, the drug being practically always taken upon a physician's order.

5. The dangerous character of amidopyrine being now established beyond any reasonable doubt, amidopyrine should be strictly banned by the medical profession.

6. When anodynes or antipyretics are indicated, we have a safe drug<sup>11</sup> in acetanilid, which should be given in therapeutic doses, best combined with caffeine and potassium citrate. (An alternative but less efficient drug would be acetphenetidin.)\*

7. By using the safer antipyretics mentioned, there is no doubt but that we will obtain greater efficiency, and rapidly eliminate granulocytopenia and other toxic phenomena now known to be caused by the dangerous pyrazolon group of drugs (amidopyrine and antipyrine).

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145 WEST SEVENTY-FIRST STREET

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## THE BACTERICIDAL AND FUNGICIDAL ACTION OF HOMOLOGOUS HALOGEN PHENOL DERIVATIVES AND ITS "QUASI-SPECIFIC" CHARACTER\*

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### II. DERIVATIVES OF ORTHOCHLOROPHENOL (WITH NOTES ON CHLORINE-FREE ALKYL PHENOL DERIVATIVES)

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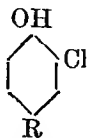
EMIL KLARMANN, SC.D., VLADIMIR A. SHTERNOV, PH.D., AND LOUIS W. GATES,  
B.S., BLOOMFIELD, N. J.

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#### INTRODUCTION

IN OUR preceding paper<sup>1</sup> we described certain regularities which we found in the relationship between the chemical constitution and the microbicidal action of aliphatic and aromatic substitution derivatives of parachlorophenol.<sup>†</sup> The present paper deals with the derivatives of orthochlorophenol in the manner of our previous work. In addition, some consideration is given herein to the halogen-free para-alkyl (and alkaryl) phenol derivatives, for two reasons; first to show what effect the introduction of chlorine into the ortho-position of the nucleus of para-alkyl phenols has upon the antibacterial action, and second to demonstrate the existence in this class of phenol derivatives of the "quasi-specific" effect previously described.

The derivatives of orthochlorophenol under consideration may be represented by the general formula:



where R is an aliphatic or aromatic substituent.

\*From the Plaut Research Laboratory, Lehn & Fink, Inc.

Received for publication, December 2, 1933.

<sup>†</sup>In this connection attention is called to another paper, dealing with bromophenol derivatives, which has been published in the meantime.<sup>2</sup>

The microorganisms used in this investigation, the methods of their cultivation, and the testing procedures were the same as described in our preceding paper.

#### PARA-ALKYL DERIVATIVES OF ORTHOCHLOROPHENOL

A perusal of Tables I to IV indicates a very similar relation between the molecular weight and the microbicidal action in the class of the para-alkyl

TABLE I

THE GERMICIDAL ACTION OF HOMOLOGOUS DERIVATIVES OF ORTHOCHLOROPHENOL UPON ORGANISMS OF THE TYPHOID COLON GROUP  
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	<i>Eberthella typhi</i>		<i>Eberthella paradysenteriae</i>		<i>Salmonella schottmülleri</i> ( <i>B. para typhosus B</i> )		<i>Escherichia coli</i>	
	I	II	I	II	I	II	I	II
o-Chlorophenol	1: 350	2.5	1: 350	2.3	1: 300	2.1	1: 300	2.3
p-Alkyl Derivatives								
Methyl	1: 1,000	6.3	1: 800	5.3	1: 700	5.4	1: 700	5.4
Ethyl	1: 2,750	17.2	1: 2,000	13.3	1: 3,500	25.0	1: 3,500	29.2
n-Propyl	1: 6,000	40.0	1: 6,000	40.0	1: 5,000	35.7	1: 4,000	33.3
n-Butyl	1:13,000	86.7	1:12,000	80.0	1:10,000	66.7	1: 5,000	38.4
n-Amyl	1:12,000	80.0	1:12,000	80.0	1: 6,000	40.0	1: 2,500	19.2
tert. Amyl	1: 4,500	32.1	1: 7,000	46.7	1: 3,000	21.4	(1: 2,500)	(17.9)
n-Hexyl	(1: 3,500)	(23.3)	---	---	---	---	---	---
n-Heptyl	(1: 2,500)	(16.7)	---	---	---	---	---	---
Aromatic Derivatives								
4-Benzyl	1: 5,000	35.7	1: 7,000	53.9	1:10,000	71.4	1: 3,500	26.9
6-Benzyl	1: 3,750	25.0	1: 5,000	38.5	1:10,000	71.4	1: 3,000	23.1
Phenol (Control)	1:140-150	1.0	1:140-160	1.0	1:140-150	1.0	1:140-150	1.0

TABLE II

THE GERMICIDAL ACTION OF HOMOLOGOUS DERIVATIVES OF ORTHOCHLOROPHENOL UPON PATHOGENIC COCCI  
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	<i>Staphylococcus aureus</i>		<i>Streptococcus (hemol. strain)</i>		<i>Micrococcus catarrhalis</i>	
	I	II	I	II	I	II
o-Chlorophenol	1: 200	2.9	1: 200	2.0	1: 300	2.0
p-Alkyl Derivatives						
Methyl	1: 600	7.5	1: 500	5.6	1: 700	4.7
Ethyl	1: 1,100	15.7	1: 1,500	15.0	1: 2,500	16.7
n-Propyl	1: 2,250	32.1	1: 3,000	33.3	1: 4,000	26.7
n-Butyl	1: 7,500	93.8	1: 8,000	88.9	1:10,000	66.7
n-Amyl	1:20,000	286.0	1:20,000	222.0	1:20,000	133.0
tert. Amyl	2:10,000	125.0	1:11,000	122.0	1: 7,000	46.7
n-Hexyl	1:40,000	500.0	1:50,000	555.0	1:50,000	333.0
n-Heptyl	1:30,000	375.0	1:30,000	350.0	---	---
Aromatic Derivatives						
4-Benzyl	1:10,000	125.0	1:13,000	163.0	1:20,000	143.0
6-Benzyl	1: 5,500	68.8	1: 8,500	94.4	1:20,000	133.0
Phenol (Control)	1:70-80	1.0	1:80-90	1.0	1:140-160	1.0

derivatives of orthochlorophenol, as was found before in the class of ortho-alkyl derivatives of parachlorophenol.

The microbicidal potency with regard to the typhoid-colon group (*Eberthella typhi*, *Eberthella paradysenteriae*, *Salmonella schottmülleri*, *Escherichia coli*)



reaches its maximum with the n-butyl derivative and declines thereafter (Table I). However, with regard to all the other test organisms (Tables II, III, and IV) comprising cocci, acid-fast bacteria and pathogenic fungi, the n-amyl

TABLE III

THE GERMICIDAL ACTION OF HOMOLOGOUS DERIVATIVES OF ORTHOCHLOROPHENOL UPON  
ACID-FAST BACTERIA  
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	<i>Mycobacterium tuberculosis (hominis)</i>		<i>Mycobacterium leprae (murium)</i>		<i>Mycobacterium smegmatis</i>	
	I	II	I	II	I	II
o-Chlorophenol	1: 200	2.2	1: 200	2.2	1: 200	2.2
p-Alkyl Derivatives						
Methyl	1: 500	5.6	1: 400	4.4	1: 500	6.3
Ethyl	1: 1,600	17.8	1: 1,600	17.8	1: 1,400	15.6
n-Propyl	1: 3,000	33.3	1: 3,000	30.0	1: 3,000	33.3
n-Butyl	1: 7,000	77.8	1: 7,000	77.8	1: 10,000	125.0
n-Amyl	1: 20,000	222.0	1: 16,000	178.0	1: 20,000	250.0
tert. Amyl	1: 10,000	111.0	1: 8,000	88.9	1: 11,000	138.0
n-Hexyl	1: 16,000	178.0	1: 20,000	222.0	1: 40,000	500.0
n-Heptyl	1: 7,000	77.8	1: 10,000	111.0	1: 16,000	200.0
Aromatic Derivatives						
4-Benzyl	1: 13,000	163.0	1: 14,000	140.0	1: 12,000	150.0
6-Benzyl	1: 10,000	125.0	1: 12,000	120.0	1: 8,000	100.0
Phenol (Control)	1: 90-100	1.0	1: 90-100	1.0	1: 90-100	1.0

or the n-hexyl derivatives are the most potent; moreover their potency is of a higher order of magnitude. The latter compound which shows a maximum potency with regard to the three pathogenic cocci and a very considerable one (though not always a maximum) against the other microorganisms studied, is comparatively very little effective against *Eberthella typhi* and also against the other three bacteria of the typhoid-colon group; but no definite quantitative data could be obtained in reference to the latter microorganisms owing to wide fluctuations in the individual experiments.

TABLE IV

THE FUNGICIDAL ACTION OF HOMOLOGOUS DERIVATIVES OF ORTHOCHLOROPHENOL  
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	<i>Monilia albicans</i>		<i>Trichophyton rosaceum</i>	
	I	II	I	II
o-Chlorophenol	1: 250	2.2	1: 200	2.2
p-Alkyl Derivatives				
Methyl	1: 750	8.3	1: 700	7.0
Ethyl	1: 2,000	22.2	1: 1,600	17.8
n-Propyl	1: 4,000	44.4	1: 3,750	41.7
n-Butyl	1: 8,000	88.9	1: 8,000	80.0
n-Amyl	1: 25,000	278.0	1: 20,000	200.0
tert. Amyl	1: 10,000	100.0	1: 10,000	90.9
n-Hexyl	1: 25,000	278.0	1: 16,000	160.0
n-Heptyl	1: 7,000	70.0	----	---
Aromatic Derivatives				
4-Benzyl	1: 14,000	156.0	1: 14,000	156.0
6-Benzyl	1: 8,000	88.9	1: 7,000	77.8
Phenol (Control)	1: 90-110	1.0	1: 90-110	1.0

Thus the "quasi-specific" effect again is in evidence, beginning with p-n-hexyl-o-chlorophenol which shows such considerable germicidal potency with regard to some microorganisms and so little of it with regard to others. The difference between the molecular weights of the compounds of maximum potency against the two groups of microorganisms into which this effect divides them, is narrow involving the step from the n-butyl to the n-amyl or n-hexyl derivative.

While there are in the series of orthochlorophenol derivatives several compounds of noteworthy germicidal and fungicidal potency, a comparison of the microbiocidal action of the individual members of this series with that of the parachlorophenol series studied previously, reveals without exception a considerable superiority of the ortho-alkyl derivatives of parachlorophenol over

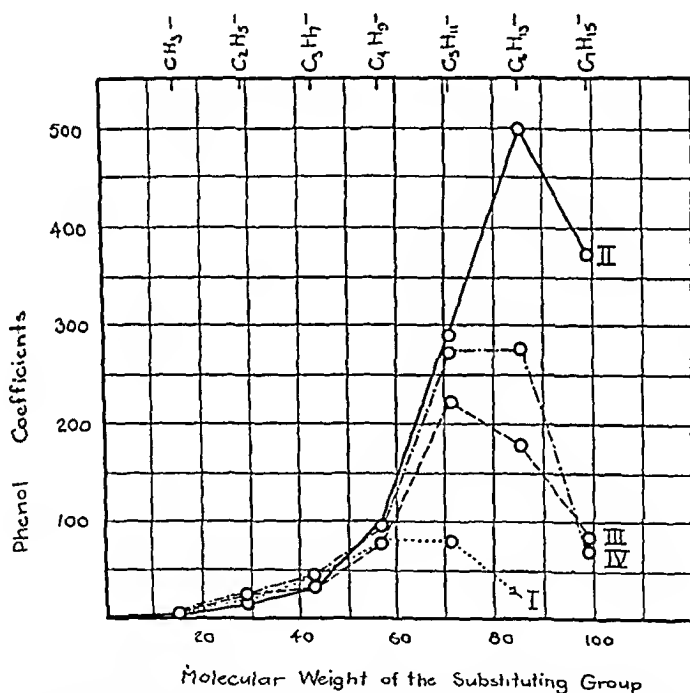


Fig. 1.—The germicidal action of para-alkyl derivatives of orthochlorophenol. Test organisms: I . . . . . *Eberthella typhi*, II ————— *Staphylococcus aureus*, III - - - - - *Mycobacterium tuberculosis* (hom.), IV - . . . . *Monilia albicans*.

the para-alkyl derivatives of orthochlorophenol. The same remarks apply to the p-benzyl-o-chlorophenol as compared with the o-benzyl-p-chlorophenol previously prepared. The o-benzyl-o-chlorophenol is less effective than the corresponding p-benzyl derivative.

For reasons of economy the discussion of Tables I to IV is limited to the above few remarks; the reader is referred to the tables themselves for further details. A graphic description of the conditions encountered in this series of compounds, illustrating the "quasi-specific" effect of the higher homologs is furnished by Fig. 1 in which the data obtained with one representative test-organism of each of the four groups studied have been used for the sake of greater clarity.

## PARA-ALKYL PHENOL DERIVATIVES (CHLORINE-FREE)

While the antibacterial properties of the homologous series of phenol derivatives have been referred to by previous investigators,<sup>3</sup> it was deemed desirable to carry out a bacteriologic investigation of the para-alkyl phenol series with the aid of representative test-organisms and using the same experimental conditions in order to ascertain the effect of substitution by chlorine in the ortho-position to the hydroxy group upon the germicidal action.\* Table V presents the results obtained in terms of minimum concentrations destructive of microbial life in ten minutes at 37° C., and the phenol coefficients calculated therefrom.

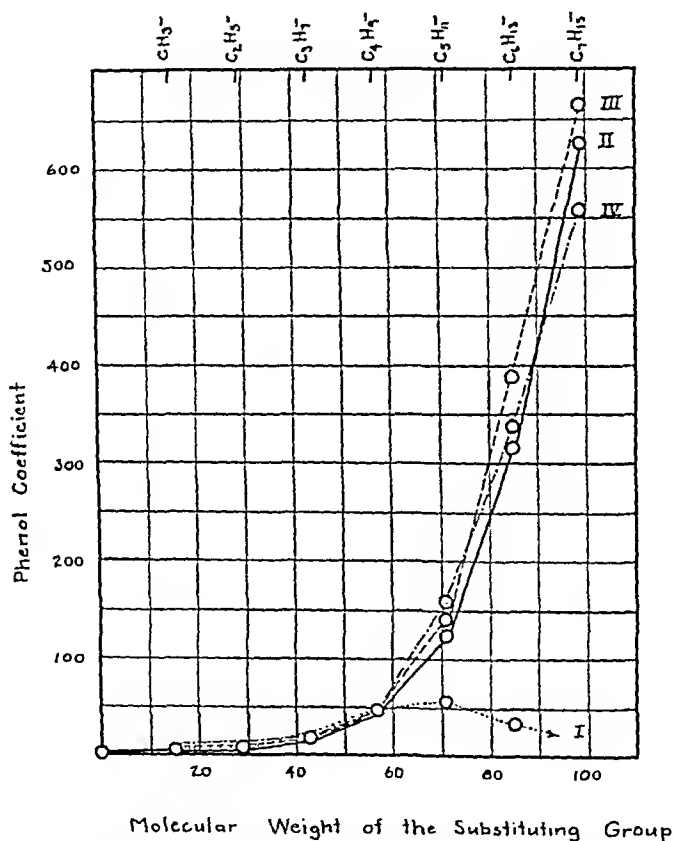


Fig. 2.—The germicidal action of para-alkyl derivatives of phenol (chlorine-free). Test organisms: I . . . . . *Eberthella typhi*. II ————— *Staphylococcus aureus*. III - - - - - *Mycobacterium tuberculosis* (hom.). IV . . . . . *Monilia albicans*.

When compared with the respective columns in Tables I to IV it shows that with the exception of the homologs with the highest molecular weights the o-chloro derivatives are on the average about twice as effective as the chlorine-free para-alkyl phenols.

The phenomenon of "quasi-specific" action observed in the several series of halogen alkyl phenol derivatives is encountered also in the series of the

\*These compounds were obtained by the reduction of the corresponding ketones by means of amalgamated zinc and hydrochloric acid; the ketones, in turn, were formed by an intramolecular rearrangement of the corresponding esters of phenol in the presence of aluminum chloride under definite conditions.

TABLE V

THE MICROBICIDAL ACTION OF PARA-ALKYL PHENOL DERIVATIVES (CHLORINE FREE)  
MINIMUM CONCENTRATIONS EFFECTIVE IN 10 MIN. (I) AND PHENOL COEFFICIENTS (II)

	<i>Eberthella typhi</i>		<i>Staphylococcus aureus</i>		<i>Mycobacterium tuberculosis (hominis)</i>		<i>Monilia albicans</i>	
	I	II	I	II	I	II	I	II
Phenol	1: 150	1.0	1: 80	1.0	1: 90	1.0	1: 90	1.0
Alkyl Derivatives								
Methyl	1: 300	2.0	1: 160	2.0	1: 200	2.2	1: 200	2.0
Ethyl	1:1,000	6.3	1: 500	6.3	1: 600	6.7	1: 700	7.8
n-Propyl	1:2,750	18.3	1: 1,300	16.3	1: 1,600	17.8	1: 1,600	17.8
n-Butyl	1:7,000	46.7	1: 3,500	43.7	1: 4,000	44.4	1: 4,000	44.4
n-Amyl	1:8,000	53.3	1:10,000	125.0	1:12,000	133.0	1:14,000	156.0
n-Hexyl	1:5,000	33.3	1:25,000	313.0	1:35,000	389.0	1:30,000	333.0
n-Heptyl	(1:2,500)	(16.7)	1:50,000	625.0	1:60,000	667.0	1:50,000	556.0

halogen-free para-alkyl phenols. Thus, with respect to *Eberthella typhi* the microbicidal potency increases at first with the increasing weight of the substituting radical, reaching a maximum in the case of the p-amyl phenol, and decreases thereafter, while with regard to *Staphylococcus aureus*, *Mycobacterium tuberculosis (hom.)*, and *Monilia albicans* the increase is continuous without the evidence of a maximum being reached in the case of the n-heptyl derivative; the same compound shows comparatively very little efficacy against *Eberthella typhi*. These conditions are illustrated graphically in Fig. 2. The similarity in the course of the curves in Fig. 1 in this and the preceding paper† is remarkable and justifies the assumption that the "quasi-specific" effect is common to different homologous series of phenolic germicides. (By way of advance information, further support is lent to this idea by the findings made in the case of the three series of monoethers of dihydric phenols.)

## TOXICITY

Using the same method as described in our preceding paper, we determined the toxicity of the p-n-alkyl compounds of the orthochlorophenol series and also of those of the series of chlorine-free p-alkyl phenols (Table VI). In the former

TABLE VI  
TOXICOLOGIC DATA  
SUBCUTANEOUS INJECTION IN MICE  
MINIMUM LETHAL DOSES IN MILLIGRAMS PER GRAM OF BODY WEIGHT

	P-ALKYL DERIVATIVES OF O-CHLOROPHENOL	P-ALKYL DERIVATIVES OF PHENOL (CHLORINE FREE)
Phenol		0.45
o-Chlorophenol	0.7	
p-Alkyl Derivatives		
Methyl	1.5	0.50
Ethyl	4.0	1.0
n-Propyl	6.0	2.0
n-Butyl	15.0	3.0
n-Amyl	20.0	5.0
n-Hexyl	>20.0	6.0
n-Heptyl	>20.0	10.0

†And also in Fig. 1 of the paper dealing with bromophenol derivatives.<sup>2</sup>

series the minimum lethal doses are almost the same as found in the o-n-alkyl parachlorophenol series studied previously; more particularly, enormously large doses of the higher homologs appear to be tolerated by the animal organism. Thus the remarks made in our previous paper as to a potential utility of some of these compounds for chemotherapeutic purposes apply as well in the case of the series under consideration.

Table VI gives also the minimum fatal doses of the compounds of the (chlorine-free) p-n-alkyl phenol series. Here, too, the toxicity decreases sharply with the increasing weight of the substituting group; i.e., as the germicidal potency increases the toxicity decreases. A comparison of the chlorine substituted and the chlorine-free homologous series discloses that the introduction of the chlorine atom into the nucleus of a p-alkyl phenol is accompanied by a sharp drop of the toxicity (and, as indicated before, by a sharp increase in microbicidal potency).

#### SUMMARY

Continuing the studies on the bactericidal and fungicidal properties of halogen phenol derivatives, we investigated the homologous orthochlorophenol series with the aid of the same methods, and using the same test organisms as described in detail in our preceding paper.

As in the parachlorophenol series previously studied, so also in the orthochlorophenol series we found a number of compounds of a remarkable bactericidal and fungicidal potency. However, when compared with corresponding compounds of the parachlorophenol series, the aliphatic and aromatic derivatives of orthochlorophenol are less effective microbicides than the corresponding compounds of the former series.

The division of the microorganisms into two classes, on the basis of their susceptibility to the selective, "quasi-specific" action of the higher homologs, as observed in the case of the compounds of the parachlorophenol series, is effected by certain orthochlorophenol derivatives as well.

The bacteriologic study of the homologous series of the halogen-free p-alkyl compounds of phenol was included in this work in order to ascertain the effect of the introduction of chlorine upon the microbicidal action. The demonstrated existence of the "quasi-specific" effect in this series of phenol derivatives (and also in other series discussed elsewhere) permits the conclusion that this effect is common to different classes of phenolic germicides.

The toxicity to mice of the compounds of the homologous orthochlorophenol series agrees very closely with that of the corresponding parachlorophenol derivatives. Thus in the series under discussion, too, the increasing weight of the substituting radical not only produces an increase in microbicidal potency (within definite limits) but also a decrease of toxicity. A comparison with the chlorine-free p-alkyl phenol derivatives shows that the introduction of chlorine into the nucleus of p-alkyl phenols brings about a very decided drop in the toxicity.

We acknowledge gratefully the valuable assistance rendered by Mr. A. Grawehr, of our laboratory staff, in the bacteriologic part of this investigation.

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## A STUDY OF COCCIDIOIDES IMMITIS\*

CORNELIA McDONALD, A.B., MONTGOMERY, ALA.

## INTRODUCTION

THE patient from whom the organism *Coccidioides immitis* was isolated was admitted to the Veterans' Administration Hospital, Alexandria, La., Sept. 12, 1931, with diagnosis of pulmonary tuberculosis. The tubercle bacilli had not been found.

Report of finding the organism *Coccidioides immitis* was made Oct. 7, 1931. The patient was put on iodide treatments. Ten days later the routine sputum examination revealed *B. tuberculosis*, as did succeeding examinations. When first seen by the writer, the lesion of coccidioidal granuloma involved a small area with a diameter of about 1.0 cm., in the pharynx. Three months later the major part of the buccal cavity was filled with discharging sinuses. The patient was discharged from the hospital Jan. 15, 1932, and died at his home Mar. 7, 1932.

The patient had spent his entire life in Louisiana, except nine months' World War service, four months of which was overseas service. He had been a gas meter mender and had suffered nasal and pharyngeal irritation from gases while at his work. In December, 1930, he had made some metal lamps from junk material and recalled the extreme irritation due to the dust from the buffing wheel. The initial lesion appeared about six weeks later.

This statement is made because infection with this organism is rare, and in this country is confined almost exclusively to the San Joaquin Valley, California; also the factors in the onset of the disease have varied greatly. The only previous case report from the South was made by Lynch<sup>1</sup> in 1920.

## GROWTH ON ARTIFICIAL MEDIA AND MICROSCOPIC FINDINGS

The organism *Coccidioides immitis* was first seen in this case in cultures and was found later in fresh and stained smears. The small lesion on the patient's pharynx had been scraped and several broth tubes inoculated. Within twenty-four hours a fluffy mass had formed which remained suspended in the clear medium for some time, but gradually it sank to the bottom of the tube. This mass was made up of a network of filaments and spores. (Fig. 3.)

Transfers were made from the culture tube to agar plates and carbohydrate broths. On the carbohydrate media the growth was rapid and abun-

\*Received for publication, January 22, 1934

dant, but no acid or gas formed. At the end of forty-eight hours slightly raised white colonies had formed on the agar plates, and later an occasional brown colony was noticed. In the colonies were found small sporelike bodies which were gram-positive, nonacid and alcohol-fast. (Fig. 5-A.) Growing from the colonies were filaments which were branched and septate and contained

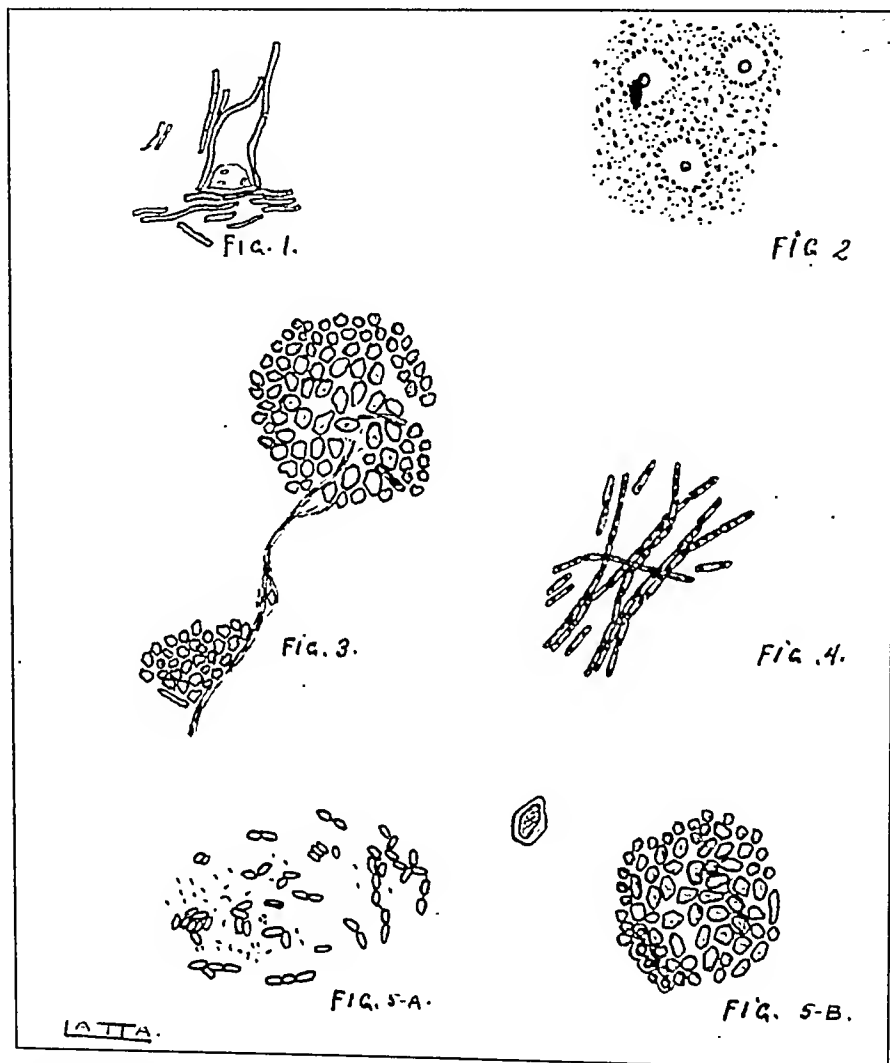


Fig. 1.—An empty spherical capsule and mesh of mycelium. (From animal tissue and culture forms.)

Fig. 2.—Double-contoured spherical forms. (From animal tissue.)

Fig. 3.—Broth. Hyphae and conidia.

Fig. 4.—Agar. Mycelium.

Fig. 5-A.—Agar. Spore forms.

Fig. 5-B.—An elliptical double-contoured form and spore forms. (From animal tissue and culture forms.)

bright, refractile vaeuolcs. (Fig. 4.) A loop of the mycelium was transferred to broth tubes and plain agar slants. In broth, characteristics were identical with former findings on this medium; on the agar slants aerial

hyphae developed after a few days and grew rapidly into a cloudy white covering. Culturally and microscopically the organism showed all the characteristics first described in detail by Wolbueh.<sup>2</sup>

Cultures from the human lesion were kept growing for six months on ordinary laboratory media. Carbohydrate broths and Sabourand's agar gave the most rapid and abundant growths. In liquid media flakes formed in the clear medium, or a grayish white, lacelike covering appeared on the surface; both forms slowly sank to the bottom of the tube. As the cultures grew old, this sediment changed in color from tan to dark brown. Broth tubes, some containing the original material, others subcultures, were allowed to evaporate in the incubator. These were transferred to the ice box for six weeks. Dextrose broth was added to the moist sediment and the tubes replaced in the incubator. The liquid changed from a dark brown to burgundy color within twenty-four hours, and a heavy network covered the surface.

On solid media the growth usually appeared as seen in agar cultures; small, discrete, white colonies, from which aerial hyphae, or deep penetrating mycelium, or both, grew. The colonies which remained discrete grew to several times the original diameter, often appeared in concentric circles with wrinkled surface and varied in color from cream to dark brown. When growing thickly, the small white colonies spread rapidly over the surface of the medium forming a creamy covering from which pure spore forms could be recovered. Aerial hyphae were most abundant, though delayed in development when grown at room temperature. Conidia, usually arranged at the end of hyphae, were observed in old cultures. Chlamydospores within the hyphae, described by Ophüls,<sup>3</sup> were found occasionally. They bore a marked resemblance to the spherical bodies recovered from the sputum and found in animal tissue.

Rixford and Gilchrist<sup>4</sup> first reported in this country the morphology of *Coccidioides immitis* as it appears in the tissues, a double-contoured spherical structure, varying from 5 to 60 microns or more in diameter. The double life cycle, spherical forms in the animal body, mycelia in artificial media, and their transformation, have been described by several writers. With a modification of the method of MacNeal and Taylor,<sup>5</sup> we studied the transformation of spherical bodies into mycelia. Fresh pus was taken from a lesion of the disease and suspended in broth. A loop of the suspension was placed on a flamed cover glass. A drop of agar was placed in a sterile hollow-ground slide. The cover glass was inverted over the well and sealed on the slide with paraffin. Several preparations were made, incubated, and examined daily. In some organisms no changes occurred, from others protoplasm flowed through the cell wall, branched and developed into a network of septate mycelium. As the culture grew old, the hyphae became clubbed, and spores formed, development previously observed in plate cultures.

#### ANIMAL EXPERIMENTATION

It was known that *Coccidioides immitis* is pathogenic for rabbits, guinea pigs, and dogs. For the purpose of studying the organism in tissues and mak-



ing certain serologic examinations it was decided to reproduce the disease coccidioidal granuloma in experimental animals.

Three guinea pigs were each given a 0.5 c.c. intravenous injection of a killed mycelial growth of *Coccidioides immitis*, suspended in 0.85 per cent sodium chloride solution. The growth had been removed from an old agar culture in which aerial hyphae had grown so densely that the appearance was that of a light cotton covering. The guinea pigs were killed three weeks later. Pathologic findings were small firm masses on the abdominal wall, pinpoint lesions on the liver, a lumbar vertebra lesion, miliary nodules on the lungs and spleen, and red and swollen testicles. In smears and cultures *Coccidioides immitis* was found.

Histologic sections were made from the spleen and testes of one of the guinea pigs. The coccidioidal bodies stained readily with methylene blue, hematoxylin-eosin, iron-hematoxylin, and with Gram stain. These double-contoured

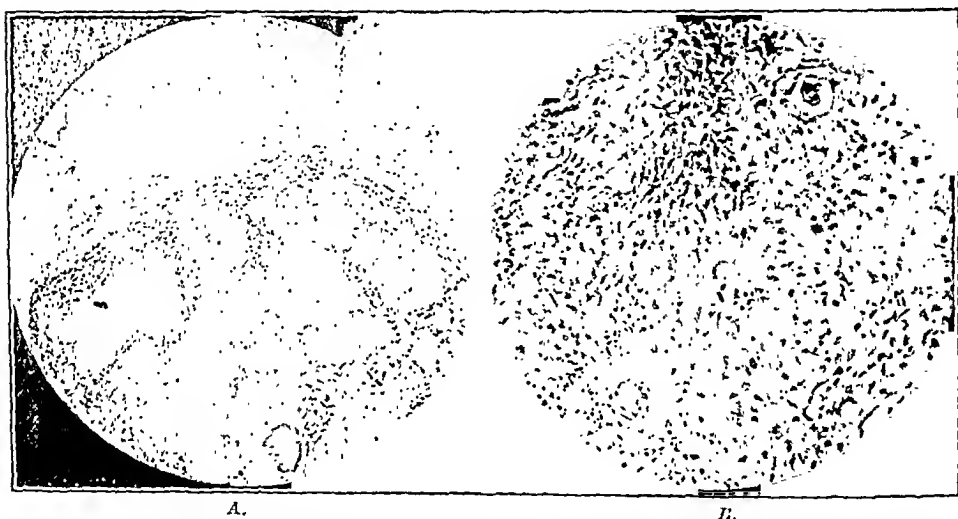


Fig. 6.—A, Liver of rabbit through calcified necrotic area and cellular infiltration showing two coccidioidal bodies in the center. Low power. B, The same two bodies and granulomatous type of cells. The foreign body at upper right is of unusual view. Inner sac dense, packed spores, one of which can be seen escaping into the open space between the walls. High dry power.

bodies varied greatly in size. No budding or mycelial growth occurred. Reproduction takes place by the formation of small intracellular spore bodies, which may be capsulated within the parent cell. The spore bodies are liberated by bursting capsule and grow out directly, either outside, or often still within old capsule, into young spherical forms.

An old agar culture of *Coccidioides immitis* from the patient's lesion was used for intraperitoneal inoculation of two rabbits. The heavy white mold was removed from agar plates, washed, and centrifuged in 0.85 per cent sodium chloride solution. The injection was made of 2.0 c.c. of the suspension of organisms, many rather elliptical forms, some round forms, and a few broken filaments.

An antigen had been prepared for use in making complement fixation tests, precipitation and agglutination tests. The patient's lesion was aspi-

rated and a loop of the pus examined for spherical forms. The exudate was allowed to dry and was extracted with alcohol and ether. A grayish white powder was recovered and dissolved in 0.85 per cent sodium chloride solution.

Mixtures of the rabbit serum and the suspension were positive in precipitation tests in dilutions of from 1:20 to 1:240 and in dilutions from 1:20 to

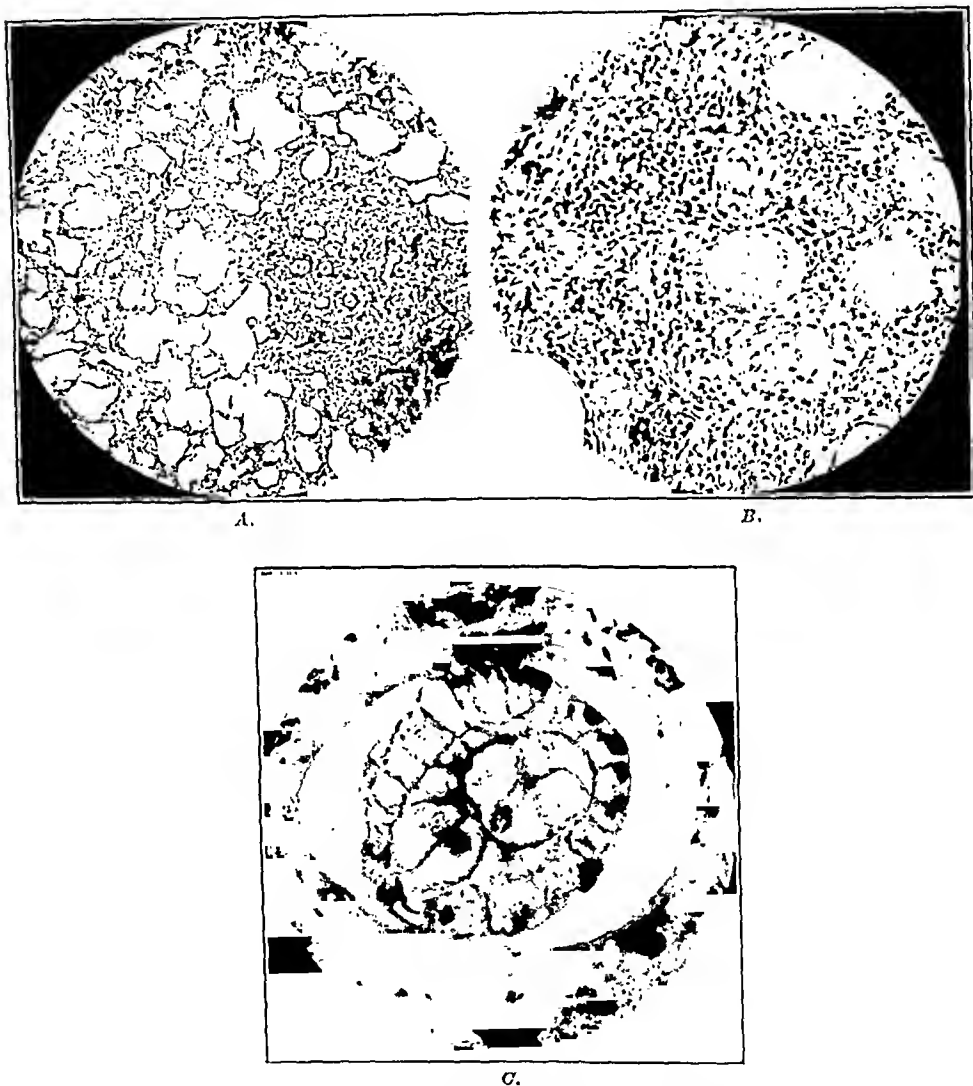


Fig. 7.—A, Cat lung. There are large granulomatous areas showing several coccidioidal bodies. Low power. B, The same bodies photographed in A. In the upper right foreign body the inner wall has ruptured and sporulating forms are free in the outer sac. Just below is a small form in which the endospores are eccentrically placed in the inner wall. Farther below there is a large body which shows vacuoles and protoplasmic content in a double-contoured wall but no endospores. In the center of the field is a coccidioidal body, which is seen in detail in C. High dry power. C, Oil immersion. The architecture of coccidioidal body near center of field Figs. A and B, spherical form, double-contoured a space between which does not stain, a vacuolated protoplasmic sac containing sporulating bodies.

1:320. The serums were negative for complement fixation tests and agglutination tests. With similar methods Davis,<sup>6</sup> who experimented with patient's serum, obtained positive complement fixation reactions. Davis and also Cooke<sup>7</sup>

had negative results with agglutination tests and positive results with precipitation tests.

After the second week, the rabbits rapidly lost weight and were killed at the end of the third week. One was examined. Nodules appeared on the peritoneal surface and about the intestines. Pearly nodules appeared on the liver; the spleen ruptured. Several of the pearly structures were removed from the liver, crushed between glass slides, moistened with 10 per cent sodium hydroxide and examined. The double-contoured bodies of *Coccidioides immitis* were found. In stained smear and cultures, the forms previously described were observed. Photographs and descriptions of the coccidioidal bodies in histologic sections from the rabbit's spleen are seen in Figs. 6A and 6B.

A very large house cat was given a 3 c.c. intraperitoneal injection from the same suspension from which the rabbit had been inoculated. Two weeks later nasal discharges were noticed. The serum was positive in precipitation tests in dilutions of from 1:20 to 1:80. The cat was killed at the end of three weeks. The lungs showed miliary lesions. The spleen ruptured. In smears from the lungs the highly refractile bodies and endosporeulating forms of *Coccidioides immitis* were found. Histologic sections of the lungs were photographed. The photographs, also descriptions, of the coccidioidal bodies under different magnifications are seen in Figs. 7A, 7B, and 7C.

The experimental animals used in the work were fully grown, healthy males. Within two weeks after inoculation with the *Coccidioides immitis* organisms a change in physical condition could be noticed, and this change was pronounced by the end of the third week. Loss of weight, loss in strength, roughness and loss of hair were characteristic. The guinea pigs became highly nervous; the rabbits and cat were sluggish and indifferent.

#### SUMMARY

1. *Coccidioides immitis* is a fungus which has a double life cycle. It reproduces by mycelium in cultures and by endogenous spore formation in tissues.

2. *Coccidioides immitis* is the cause of a definite infectious disease, coccidioidal granuloma, in man.

3. The disease can be reproduced in the guinea pig, rabbit, and cat by intravenous and intraperitoneal injections of *Coccidioides immitis* organisms.

4. The serum of animals in which coccidioidal granuloma has been reproduced gave positive reactions in precipitation tests, negative reactions in the complement fixation and agglutination tests.

5. The organisms of *Coccidioides immitis* are found in pus, sputum, tissues of infected animals and can be recovered by culture and animal inoculation.

While the major part of this work was done after work hours, I wish to express appreciation for encouragement received from Dr. L. A. Walker, clinical director, who suggested that I write this report for publication; and Dr. J. B. Latta, pathologist, who made the excellent drawings of forms of *Coccidioides immitis* recovered from cultures.

Acknowledgment is made to the Veterans' Administration, Washington, D. C., for permission to publish this paper and for recognition of the work in the *Medical Bulletin*.<sup>8</sup>

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## OBSERVATIONS ON THE GONOCOCCIDAL ACTION OF MALLOPHENE IN URINE\*

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RUSSELL D. HERROLD, CHICAGO, ILL.

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IT IS well known that the bactericidal action of antiseptics in urine does not parallel the action in water at the same dilution. As a rule a greater concentration is needed in urine than in water to obtain the same bactericidal action. Therefore, preference should be given to urines as a medium for dilution of the antiseptic for bactericidal tests when the antiseptic is proposed for clinical use in the treatment of infections of the urinary tract.

Gonococci are killed quickly in highly acid or highly alkaline urines. Therefore, when observations are not made near neutrality, careful controls should be made of the urine without the addition of antiseptics.

It may be seen from Table I that gonococci were killed in twenty minutes in dilution of 1-2,000 and 1-3,000 of mallophene in urine in all hydrogen ion concentrations from 4.9 to 7.0. In this observation the hydrogen ion readings were made before the urine was sterilized by boiling so that the actual hydrogen ion concentration in the test solutions were somewhat higher than recorded in Table I. A four-plus growth was obtained in the control of urine 6 whereas there was no growth in this urine containing the mallophene solutions.

Table II demonstrates that mallophene in a dilution of 1-4,000 did not kill the gonococci in twenty minutes in urines near the neutral point, but there was no growth after twenty minutes in the more acid urines containing mallophene. At least part of the bactericidal action in the urines with a high hydrogen ion concentration should be attributed to acidity so that it would seem that mallophene in a dilution of 1-3,000 is necessary to kill gonococci within twenty minutes near neutrality.

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\*Received for publication June 29, 1934.

Urine does not seem to have a distinct bactericidal action between pH 6.4 and 7.0. Below 5.4 all urines exhibit some bactericidal action to the gonococcus, but between 5.4 and 6.4 all urines are not equally bactericidal in proportion to acidity, and it is apparent that there are other factors in individual instances that influence bactericidal action in addition to acidity.

TABLE I  
GONOCOCCIDAL TEST OF MALLOPHENE ADDED TO URINE\*

DILUTION OF MALLOPHENE	URINE 1 pH 4.9	URINE 2 pH 5.4	URINE 3 pH 5.7	URINE 4 pH 5.8	URINE 5 pH 6.2	URINE 6 pH 7
1-2,000						
0.5 c.c. 1-100 Mallophene and 9.5 c.c. Urine	0	0	0	0	0	0
1-3,000						
0.5 c.c. 1-150 Mallophene and 9.5 c.c. Urine	0	0	0	0	0	0
Control (Urine Without Mallophene)	1+	2+	2+	2+	3+	4+

\*Subcultures to solid mediums from various urines 20 minutes after addition of gonococcus suspension in urine 6.

TABLE II  
MALLOPHENE 1-4,000 ADDED TO BOILED URINE AND GONOCOCCIDAL TESTS MADE

SPECIMEN	pH OF BOILED SPECIMEN	GROWTH AFTER SUBCULTURES		pH OF FRESH SPECIMEN
		5 MIN.	20 MIN.	
1	5.6	0	0	4.9
2	5.7	1+	0	5.1
3	6.1	0	0	5.7
4	6.6	1+	0	6.3
5	6.8	2+	1+	6.5
6	7.6	4+	3+	6.8
7	7.8	4+	3+	6.8
Control Urine Without Mallophene 5	6.8	4+	4+	6.5

TABLE III  
GONOCOCCIDAL TEST WITH MALLOPHENE EXCRETED IN URINE OF PATIENT WITH NORMAL URINARY TRACT: SUBCULTURES 25 MINUTES AFTER GONOCOCCI WERE ADDED\*

SPECIMEN	pH	GROWTH AFTER SUBCULTURE
1 Before Mallophene (Control)	5.4	0
1A 1 hr. After Two Mallophene Tablets	5.4	0
1B 2 hr. After Mallophene	5.6	0
1C 2 hr. 40 min. After Mallophene	6.0	2+
1D 3 hr. 10 min. After Mallophene	7.0	3+
2 Control	5.2	0
3 Control	5.6	3+
4 Control	6.7	4+

\*One tablet of mallophene was given the patient at the time control specimen 1 was voided. One dram of sodium bicarbonate was given the patient after specimen 1B was voided.

An experiment was made to determine the antiseptic action of urine after the oral administration of mallophene and at various hydrogen ion concentrations. It may be noted in Table III that acidity alone killed the gonococci at a pH of 5.4 and below while there was growth in the presence of mallophene at a pH of 6.0 and above.

TABLE IV

BACTERICIDAL TESTS WITH EXCRETED MALLOPHENE URINES IN PATIENTS WITH GONORRHEA

PATIENT	pH	DURATION AFTER VOID- ING UNTIL INOCULA- TION	RESULTS	DURATION AFTER VOID- ING UNTIL INOCULA- TION	RESULTS	DURATION AFTER VOID- ING UNTIL INOCULA- TION	RESULTS
1	5.1	10 min.	5 colonies	42 min.	0		
2	5.2	20 min.	50 colonies	30 min.	30 colonies	1 hr.	2 colonies
3	6.6	0	50 colonies	1 hr.	25 colonies		

Table IV illustrates the results by culture of the inoculation of shreds of the urine at various intervals. These patients had active gonococcus infections, and mallophene was administered by mouth. It is possible that gonococci in shreds are more protected from antiseptic action since in one instance there was moderate growth after thirty minutes with a hydrogen ion concentration of 5.2.

While the above experiment does not indicate a high germicidal action of excreted mallophene in urine, clinically, in some instances of posterior infection, there was clearing of the second urine prompt enough following the administration of mallophene to indicate that there is favorable action in posterior gonococcus urethritis, particularly during the course of disease when anteroposterior irrigations are not likely to be well tolerated. However, the excretion of antiseptics is less likely to be followed by complications during this stage of the disease than retrograde irrigations through the urethra. It is possible that benefit from the antiseptic excreted in the urine may be partially due to cellular stimulation since the dye type of antiseptics is said to have increased permeability.

# THE EFFECT OF ALPHA DINITROPHENOL (1-2-4) ON BLOOD CHOLESTEROL IN MAN\*

LUTHER F. GRANT, M.D., AND PURCELL G. SCHUBE, M.D., BOSTON, MASS.

ALPHA dinitrophenol (1-2-4) is a crystalline solid. It is slightly soluble in water, and more so in ether or alcohol. In ethylene or propylene glycol it will make a 2 per cent solution, and when dissolved in water to which one-half its weight of sodium bicarbonate has been added, it makes a 3 per cent solution. It has a yellow brown color at a  $P_H$  of about 8.1 and becomes colorless at a  $P_H$  of about 2.5. It has a melting point of  $114^{\circ}$  C.

## SURVEY OF LITERATURE

In 1916 Barral and Martin<sup>1</sup> reported that when alpha dinitrophenol was inhaled in the form of a vapor or dust there frequently resulted headache, dizziness, vomiting, fever, and occasionally death. In 1917 Lutz and Baume<sup>2,3</sup> studied the toxicology of the drug and its distribution in the organs and tissues. In 1918 Warthen<sup>4</sup> and Merklen and Malloizel<sup>5</sup> and in 1919 Quignard<sup>6</sup> reported additional studies upon this chemical substance. Interest in alpha dinitrophenol then lagged somewhat until 1931 when Magne, Mayer and Plantefol<sup>7</sup> published their studies which were closely followed by those of Cutting and Tainter<sup>8</sup> in 1932. Since then Magne, Mayer and their collaborators<sup>9</sup> and Cutting and Tainter<sup>10</sup> have published a number of interesting papers upon this substance.

In none of these studies has the effect of alpha dinitrophenol on blood cholesterol been reported. Inasmuch as this latter substance is of some importance in metabolism, it was felt that such a study was justified.

## PHYSIOLOGIC PROPERTIES

The physiologic properties of alpha dinitrophenol can be enumerated as follows:

1. Alpha dinitrophenol, when given to pigeons, rabbits, rats, cats, dogs, and men, in doses ranging between 3 and 40 mg. per kilogram of body weight, by any route, produces a remarkable increase in body temperature, up to  $6^{\circ}$  or  $7^{\circ}$  C., in conjunction with marked respiratory stimulation.
2. The maximum febrile response occurs about one hour after injection or several hours after oral administration.
3. The increased temperature can occur independently of the skeletal muscles or central nervous system.

\*From the Psychiatric Clinic, Boston State Hospital.  
Received for publication, January 17, 1934.

4. The temperature is not influenced by full doses of ergotamine, adrenalectomy or thyroidectomy.
5. The respiratory stimulation may occur independently of the pyrexia.
6. The pulse rate is increased.
7. The blood pressure shows no consistent change.
8. The nitrogen excretion is less than the nitrogen intake.
9. The organic acid excretion is not increased.
10. The weight is decreased.
11. The metabolic rate is increased as much as from 25 to 50 per cent.
12. Recovery, if the dose is not fatal, requires four or more hours.
13. Death may occur as a result of direct circulatory depression, hyperpyrexia, acidosis or mioxemia, dependent on the dose, rate of injection, etc., of drug.

#### METHOD

Fourteen patients were selected at random from a relatively large group for the study. Of these one had to be dropped because of uncooperativeness. Blood cholesterol estimations were obtained on each of the patients once a week for a period of three weeks before the administration of the drug. In this manner a fairly satisfactory cross-section of the existing blood cholesterol and its variations in each of the patients was obtained. The greatest range was 30 mg. per 100 c.c. This greatest range of variation of cholesterol was used in each case of this group as the "normal" variation for that individual. It was felt that the greatest variation and not the average variation should be used in order to eliminate the existence in our results of individual false positive findings.

The patients were given alpha dinitrophenol, gr. 5, by mouth, daily, for a period of twenty days. The weight and blood cholesterol estimations of these individuals were obtained before, during, and after the administration of the chemical compound. The method of estimation of blood cholesterol was that devised by Schube.<sup>11</sup> The results obtained were compiled in Tables I and II.

#### RESULTS

In this group of cases used for study the only physical pathology clinically observed was valvular heart disease (Case 6) and syphilis (Case 11). From a study of the data obtained it was felt that this pathology did not alter the value of the results. The age range of the cases was fairly extensive (from twenty-two to fifty-nine years) but this, too, did not seem to affect the results in any significant manner. The weight of each of the individuals in this series was considered to be within normal limits for that subject at the beginning of the study. After the administration of the alpha dinitrophenol in three subjects (Cases 10, 11, 14), there were definite gains of 5, 12, and 7 pounds, respectively. The largest definite loss of weight was 4 pounds in each of the two subjects (Cases 5, 8). In the remainder of the cases, although the weight fluctuated in some instances, it remained essentially at its original level (Table



TABLE I

CASE	AGE	C = BL. CHOL.		DURATION IN DAYS										Alpha dinitrophenol started—gr. 5 daily				Alpha dinitrophenol stopped			
		W = WEIGHT		0	1	7	14	20	21	23	26	30	33	37	40	41					
1	35	C		125	90	102	105		166	150	133	125	125	127	100	53					
2	29	W			166	140			150	125	86	130	105	126	166						
		C		127	125	122	122		133	150	160	143	181	142							
4	59	W		142	112	95	86		209	157	181		166	122	68	116					
5	26	C		125	80	110	95		142	200	250	126	125	118	90	90					
6	22	W		118	166	158	163		136	100	80		180	139							
7	56	C		140	100	105	86		130	125	130	141	80	95	100	100					
8	29	W		137		94	117		124	153	107		153	134	100	83					
9	32	C		139	87	95	95		214	166	150	144	118	137	86	95					
10	39	W		158	105	79	96		171	158	160	162	100	163	148	107					
11	57	C		172	83	82	74			172	77	132	95	184							
12	50	W		132	125	136	130		140	230	160	132	124	132	111	133					
13	34	C		124	140	130	145		187	150	83	124	118	125	100	100					
14	33	W		120						121				128							

1). These observations are opposed to other reports upon the effect of alpha dinitrophenol upon body weight.

The blood cholesterol in each of these cases, irrespective of alterations in the weight, showed a deviation from normal. This deviation was usually in a positive direction. The range of this deviation was not the same for all cases. In some the blood cholesterol, after a preliminary rise, dropped below "normal" before stabilizing itself within its original range (Table I).

The blood cholesterol values during the administration of alpha dinitrophenol are compared with the average normal blood cholesterol values in Table II. The largest positive change was 155 mg. and the smallest 13 mg. Of the group, four showed an increase in blood cholesterol of 100 mg. or more; seven had values of from 33 to 100 mg.; and two had values of 33 mg. or less. The largest rise occurred on the first day in five cases, on the third day in four cases, and on the thirteenth day in three cases. The largest negative change was -82 mg.

TABLE II

CASE NO.	AVERAGE NORMAL CHOLESTEROL	BLOOD CHOLESTEROL DURING ALPHA DINITROPHENOL—MG. PER 100 C.C.							DIFFERENCE OF AVERAGE
		HIGHEST VALUE			LOWEST VALUE			AVERAGE	
		AMT.	DIFF.	DA.	AMT.	DIFF.	DA.		
1	99	166	67	1	100	1	20	139	40
2	153	166	13	13	86	-67	6	129	-24
4	123	181	58	13	133	-	-	155	32
5	98	209	111	1	68	-30	20	156	58
6	95	250	155	6	70	-25	20	161	66
7	162	180	18	13	80	-82	6	124	-38
8	97	130	33	1	80	-17	13	112	15
9	106	153	47	3	100	-6	20	127	21
10	92	214	122	1	86	-6	20	147	55
11	93	166	73	3	100	7	13	149	56
12	80	127	47	3	77	-3	6	100	20
13	130	230	100	3	111	-19	20	153	23
14	138	187	49	1	83	-55	6	124	-14

and the smallest was -3 mg. Of these negative changes, three were -50 mg. or more; two were from -25 to -50 mg.; two were from -15 to -25 mg.; and three were from 0 to -15 mg. The largest negative values occurred on the sixth day in four cases, on the thirteenth day in one case, and on the twentieth day in five cases. Thus in most of the cases, there is an initial elevation of blood cholesterol, then a drop to below the original level followed by a gradual return to "normal."

The largest average positive change was 66 mg. and the largest average negative change was -38 mg. There were ten cases with positive average values which ranged from 15 to 66 mg., four of which were 50 mg. or more, one between 40 and 50 mg., one between 30 and 40 mg., and four between 20 and 30 mg. There were three cases with negative average values, -14 mg., -24 mg., and -38 mg.

From a study of these figures and Tables I and II it can be stated that the blood cholesterol is altered during the administration of alpha dinitrophenol. We are unable to correlate or explain this alteration. Further studies are being conducted in an attempt to do these things.

## SUMMARY

Alpha dinitrophenol was administered by mouth to 13 adult men of normal weight. The weight and blood cholesterol values of these individuals were studied and presented.

NOTE: No cutaneous lesions or toxic symptoms were observed.

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## UROBILINURIA—FALSE EHRlich REACTION CAUSED BY PYRIDiUM MEDICATION\*

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J. WATTS FARTHING, M.D., AND JAMES S. P. BECK, M.D., PHILADELPHIA, PA.

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RECENTLY it was demonstrated by one of us in a patient receiving pyridium (Phenylazo-2-6-diamino-pyridine monohydrochloride—Merek and Co.) by mouth that this drug causes a false positive urobilinuria reaction when tested with Ehrlich's reagent (paradimethylaminobenzaldehyde). The fact that the dilution units were much above the highest reported pathologic urobilin findings prompted an investigation of the probable cause of this result. When pyridium was dissolved in water and tested with the acid solution of Ehrlich's reagent it was found to yield the characteristic red color of urobilin.

Aqueous and urobilin-free urine solutions of pyridium were tested in varying dilutions with Ehrlich's reagent. It was possible to detect a color change that would have been called "positive" in dilutions of either solution up to 1 part in 75,000. Goerner and Haley<sup>1</sup> have shown that the concentration of pyridium in the urine of a patient receiving 0.6 gm. by mouth daily is upward of 1 part in 3,000. The addition of HCl in the same concentration as is used in Ehrlich's reagent (1 part concentrated to 2 parts water) caused the urine or water solution of the dye to assume the characteristic color change. The change in color on testing with Ehrlich's reagent, then, is due merely to the change in  $P_H$  of the solution of the aniline dye, pyridium. Alkalinization of the solutions causes the color to change to yellow, with the formation of a crystalline precipitate. On microscopic examination this precipitate is seen to be composed of needlelike crystals.

L. D. Scott<sup>2</sup> recently proposed a modification of the Ehrlich test, which he claims will not give false positive reactions due to drugs excreted by the kidneys. He recommends the use of butyl alcohol to extract the coloration caused by the reaction between urobilin or urobilinogen and Ehrlich's reagent. He states, "A measured amount of urine is treated with an appropriate amount of a specially prepared solution of paradimethylaminobenzaldehyde. After being allowed to stand for a given time, the mixture is heated under standard conditions in a boiling water-bath for a short period, and after cooling the coloration so produced is extracted with amyl or butyl alcohol. This extracted red coloration, which is practically free from foreign material and

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\*The William Pepper Laboratory of Clinical Medicine, University of Pennsylvania, Philadelphia.

Received for publication, January 5, 1934.

other interfering colors, is then compared in the colorimeter against a standard ferrie thioeyanate solution almost identical with that employed by van den Bergh for the estimation of bilirubin in blood." Aqueous and urine solutions of pyridium were subjected to the test as he specified. The dye itself is extractable with butyl alcohol and consequently will give the same characteristic red color in the extract. That the color is due entirely to the acid was made apparent here also by the substitution of HCl for the Ehrlich's reagent.

When tested with Schlessinger's urobilin reagent, pyridium did not give a positive reaction.

The fact that the pyridium on excretion via the genitourinary tract is not chemically identical with the compound as ingested was ignored in our laboratory experiments. We first showed that the excreted product did give a positive reaction. Also, Goerner and Haley<sup>1</sup> demonstrated that by colorimetric comparison the ratio of the pure drug to the excretant is 30:33.5. The color reaction is the only thing of interest here, and the two substances are almost identical in that respect.

Urines which were shown to contain urobilin by both Ehrlich's and Schlessinger's tests were treated with HCl alone, and the color change did not occur. It is, therefore, quite easy to detect this drug reaction by the addition of HCl. We have made it routine that all urines which come to our laboratory and are shown to contain urobilin by Ehrlich's test be tested for color changes on the addition of HCl alone.

#### SUMMARY

The laboratory report of unusually high concentration of urobilin in a patient's urine led to the discovery that pyridium excreted in the urine gives a positive reaction with Ehrlich's reagent.

This positive reaction, a red or pink color, was found to be present in dilutions of the drug well above that in which it is commonly excreted in the urine.

The pink color was shown to be due entirely to the acid in the solution of Ehrlich's reagent.

Scott's modification of the test failed to remove this false reaction, in that the dye itself is extractable in butyl alcohol.

Schlessinger's test for urobilin does not give a positive reaction with pyridium.

This false reaction may be detected by treating all urines which are positive to Ehrlich's test with HCl alone. A pink or red color with acid indicates the presence of the dye. Discontinuing the pyridium for three or four days<sup>3</sup> is apparently the only means of obtaining a true test for urobilin with the Ehrlich reagent.

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# LABORATORY METHODS

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## A COMPARATIVE STUDY OF THE KELLY TEST AND THE FRIEDMAN MODIFICATION OF THE ZONDEK-ASCHHEIM TEST FOR PREGNANCY\*

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H. R. HULPIEU, PH.D., J. H. WEATHERBY, PH.D., AND C. G. CULBERTSON, M.D.,  
INDIANAPOLIS, IND.

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### INTRODUCTION

THE influence of the anterior pituitary hormone on the immature ovary and the application of this influence in the diagnosis of pregnancy has been described by Zondek and Aschheim.<sup>1</sup> Since the publication of their paper a number of modifications of their technic as well as several other tests have been described in the literature. Among the latter, that of Kelly<sup>2</sup> is one of the more recent. This test depends upon the fact that urine from pregnant women will cause the premature opening of the vaginal orifice of immature rats, whereas, normally the vaginal orifice remains closed until sexual maturity is reached. In view of the fact that the test as described by Kelly suggested several possible advantages as compared with the Friedman<sup>3</sup> modification of the Zondek-Aschheim test it seemed desirable to make a comparative study of the two tests under similar conditions.

### METHODS

The technic followed in the test in which rats were used was basically the same as that proposed by Kelly; but, instead of giving two 5 e.e. injections, one in the morning and one in the afternoon of the same day, it was found satisfactory to give one 2½ e.e. injection on each of two successive days. The final examination of the rats was made at the end of ninety-six hours. This modification was tested in a number of preliminary experiments and found satisfactory. The urine was made neutral to litmus, filtered, and warmed approximately to body temperature before the intraperitoneal injections were made. Catheterized specimens were used in most instances. The technic used in the Friedman test consisted of the intravenous injection into rabbits of from 10 to 15 e.e. of neutralized urine on each of two successive days. The rabbits were killed forty-eight hours after the first injection and the ovaries examined. Both tests were made

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\*From the Department of Pharmacology and Central Laboratories, Indiana University School of Medicine and Hospitals.

Received for publication, December 28, 1933.

on the same specimen, but were made independently of each other so that the results of one test could not influence the decision on the other test. The results of both tests were later compared with the final diagnoses as recorded in the hospital records.

### RESULTS

The results obtained from the comparative study of the two pregnancy tests are shown in Table I. A total of 59 cases were studied. According to subsequent histories as shown in the hospital records, there were 19 cases of pregnancy and 40 cases in which there was no pregnancy. It will be seen in Table I that the Friedman test showed 20 positives, whereas the Kelly test showed 21 positives. The error in the Friedman test was found to be due to the retention of at least part of the placenta after an abortion, which is known to give a positive result. One of the two errors in the Kelly test was made on the same specimen which gave the error in the Friedman test and is subject to the same explanation. The other error occurred in a case in which the patient was suffering from an ovarian cyst. The Friedman test on this same specimen was negative. It is generally accepted that the Friedman test depends upon the presence in pregnancy urine of anterior pituitary hormone (anterior pituitary-like hormone, Collip, et al.<sup>4</sup>). The Kelly test, however, is apparently due directly to the increased secretion of the female sex hormone by the rat as a result of the stimulation of its ovaries by the anterior pituitary hormone found in pregnancy urine. Also, it is well known that the injection into an immature rat of female sex hormone alone will cause the premature opening of the vaginal orifice.

TABLE I  
COMPARISON OF RESULTS OBTAINED FROM THE TWO LABORATORY TESTS WITH THE  
CLINICAL FINDINGS IN 59 CASES

	FRIEDMAN TEST	KELLY TEST	CLINICAL FINDINGS
Positive	20*	21*	19
Negative	38	29	40
Animals died; test not repeated	1	9	
Totals	59	59	59

\*See text.

In certain pathologic conditions of nonpregnant women sufficient female sex hormone may be excreted in the urine to produce a premature opening of the vaginal orifice in the rat, and thus give a false positive in the Kelly test. Perhaps the second error in the Kelly test was the result of increased secretion by the cystic ovaries so that sufficiently large quantities of the female sex hormone were excreted to bring about the changes observed. The absence of abnormally large amounts of anterior pituitary hormone resulted in a negative Friedman test. However, there were several other cases of ovarian cyst in which negative results were obtained with the Kelly test. Perhaps in these latter cases the cysts were not of the type which cause an increased secretion of the female sex hormone. The 38 negative results from the Friedman test were correct, and one

experimental animal died in a test which was not repeated. The 29 negative results from the Kelly test were correct, and nine pairs of rats died in tests which were not repeated.

The earliest pregnancy to give a positive test was one in which the last menstrual period began approximately one month before the test was made. Another case gave positive results approximately six weeks after the onset of the last menstrual period. There were several cases of pregnancies of two months' duration, and various others ranging up to seven months' duration.

In connection with the Friedman test it was observed in three cases that rabbits which were probably too young gave false negative results; positive results were obtained upon repetition of the test with older animals. In each of these cases the rabbit was of a rather large strain developed for meat purposes, and was as large as rabbits considerably older but of a smaller strain. It is quite probable that these young rabbits would show positive results from pregnancy urine provided sufficient time was allowed—more than 48 hours. It seems evident that fully mature animals will give a positive reaction in much less time than is required for younger animals, and for that reason are preferable. In all cases in which animals were used which were known to be mature the results agreed with the clinical findings.

In one case which had been previously diagnosed as hydatidiform mole both the Friedman and the Kelly tests gave negative results, which were contrary to those expected. However, upon reexamination it was found that the mole had sloughed, which fact accounts for the results obtained.

The Kelly test has several advantages when compared with the Friedman test, as pointed out by Kelly.<sup>2</sup> They are: ease of handling the animals, economy in purchasing or raising rats as compared with rabbits, and the fact that it is unnecessary to kill or operate on the test animals to observe the results. However, the authors found the following disadvantages: approximately double the time is required, certain pathologic conditions in which an abnormally large amount of female sex hormone is excreted in the urine may give false positive results, and, unless adequate steps are taken to prevent it, the mortality rate among rats is likely to be high as a result of the intraperitoneal injections.

#### CONCLUSIONS

1. The Kelly test for pregnancy was found to compare favorably in accuracy with the Friedman modification of the Zondek-Aschheim test in a series of 59 cases, the results having been checked against the clinical findings in each case.
2. It appears probable that certain pathologic conditions affecting the ovaries directly may give a false positive reaction in the Kelly test, whereas the Friedman test will be negative under the same conditions.
3. Since the injections of urine in the Kelly test are made intraperitoneally, contaminated specimens are more apt to kill the experimental animals than is usually true when the injections are made intravenously, as in the Friedman test.



4. The Kelly test was found to offer no important advantage over the Friedman test except in the lower cost and the greater ease of handling the smaller animals.

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## A MICROMETHOD FOR THE ESTIMATION OF HEMOGLOBIN\*

### MODIFICATION OF THE BENZIDINE REACTION

T. V. LETONOFF, M.S., PHILADELPHIA, PA.

THE blue color obtained when benzidine, acetic acid, and hydrogen peroxide are added to hemoglobin may be used as a measure of its concentration. On the basis of this reaction a microcolorimetric hemoglobin method has been devised. While this work was in progress, the method of Bing and Baker<sup>1</sup> appeared, which was a modification of a less desirable method of Wu.<sup>2</sup> These authors did not attempt to standardize the blue color but used the port wine color obtained on standing in the presence of rather strong acetic acid.

The present paper reports briefly the conditions under which the blue color may be used as a quantitative measure of hemoglobin. The method has certain advantages for with a very simple benzidine purification a preparation is obtained which gives straight line proportionality between color development and hemoglobin concentration. This proportionality was not obtained originally with the Bing and Baker method but Bing<sup>3</sup> has apparently corrected this by further purification. Also a permanent artificial standard is described which should aid in routine estimations where it is not convenient to do oxygen capacities for a standard hemoglobin solution. The time for color development is decreased to thirty-five minutes.

#### SOLUTIONS

*Benzidine.*—Purification: Add 5 gm. of a good grade benzidine base to 1 liter of water in a 2 liter beaker and cover with a watch glass. Dissolve by boiling for a short period. Add 2 or 3 gm. of carbon black (Norit) and boil for ten minutes. Filter while hot through two layers of filter paper, taking care that all the carbon is removed. Cool in ice water or allow to remain overnight in the ice box. Filter the solution through a Buchner funnel and dry in a desiccator kept in the dark. Preserve the dry benzidine in a brown bottle.

\*From the Laboratories of the Division of Metabolic Diseases of the Philadelphia General Hospital.

Received for publication, January 17, 1934.

Solution, 0.02 per cent: Weigh 0.100 gm. of the powdered benzidine on an accurate balance. Transfer to a 1 liter beaker, add 500 c.c. water and cover with a watch glass. Dissolve by boiling for a very few minutes. Cool and dilute to 500 c.c. in a volumetric flask. Filter and preserve in a brown bottle. It is stable for at least two months.

Acetic acid, 0.25 per cent: Prepare 1 per cent acetic acid by pipetting accurately 1 c.c. of glacial acetic acid into a 100 c.c. volumetric flask and diluting to mark. If there is any doubt about the strength of the glacial acetic acid, check the 1 per cent solution by titration (10 c.c. of acid requires 16.67 c.c. of N/10 NaOH, using phenolphthalein as indicator).

Prepare 0.25 per cent acetic acid as needed by accurately diluting 1 volume of the 1 per cent acetic acid with three volumes of water. (The 0.25 per cent acetic acid does not keep well because of mold growth.)

Hydrogen peroxide, 1 per cent: Accurately pipette 1 c.c. of 30 per cent hydrogen peroxide into a 50 c.c. graduated cylinder. Dilute accurately to 30 c.c. This may be preserved, in an electric ice box for a month, providing it is exposed only while being used. The 30 per cent hydrogen peroxide should be checked occasionally by titrating the 1 per cent solution with permanganate. (To a flask add 2 c.c. of 1 per cent hydrogen peroxide, about 10 c.c. of water, and 10 c.c. of 10 per cent sulphuric acid. Titrate to first pink color with N/10  $\text{KMnO}_4$ . Per cent hydrogen peroxide =  $T \times 0.085$ , where T equals c.c. permanganate titration.) Correct if not 1 per cent.

It is important that the three solutions above be accurately made as a moderate variation in their strength appreciably affects the color development. The distilled water should be free of ammonium salts, sulphates, and chlorides, and if not should be redistilled.

*Artificial Standard.*—Ferrie ammonium sulphate, 1 per cent: Weigh accurately 1 gm. of clean, clear crystals of the salt. Dissolve in water and dilute to 100 c.c. in a volumetric flask.

Potassium ferrocyanide, 1 per cent: Prepare accurately in same manner as the ferrie ammonium sulphate.

Gum ghatti, 1 per cent: Add 1 gm. to 100 c.c. of warm water and allow to dissolve overnight. Filter through 2 or 3 layers of gauze.

To a 500 c.c. volumetric flask add exactly 6.5 c.c. of the 1 per cent potassium ferrocyanide solution. Dilute with water to about 450 c.c. Add 5 c.c. of the 1 per cent gum ghatti solution. Add exactly 10 c.c. of the 1 per cent ferrie ammonium sulphate solution. Dilute to mark and mix. Allow to stand forty-eight hours before using. This solution is kept in the ice box. It is very stable and solutions over one year old have maintained their original color concentration. Under conditions of repeated experiments in our laboratory this solution is equivalent to the color produced by a concentration of 10 gm. of hemoglobin per 100 c.c. of blood when analyzed by the method proposed. One may check the strength of the artificial standard by doing oxygen capacities on blood. If necessary to modify the strength of the standard do so by adding more or less water while keeping the other constituents as directed.

## METHOD

Prepare a 1 to 1,000 dilution of blood. This may be accomplished in any way suitable to conditions. Using the ordinary hemoglobin pipette (carefully calibrated), deliver 0.02 c.c. into exactly 20 c.c. of water. From an ordinary red blood cell counting pipette which dilutes 1-200,\* 0.2 c.c. is added to 0.8 c.c. of water in a test tube. If only a small amount of blood is available, a special capillary pipette may be prepared to deliver 1 c.mm. of blood directly into 1 c.c. of water in the test tube. For larger amounts of blood the Folin micro sugar pipette, calibrated for 0.1 c.c. is convenient, diluting to 100 c.c.

Transfer 1 c.c. of the 1-1,000 dilution to a clean dry test tube. (A 50 per cent solution of acetic acid is convenient to wash previously used tubes.) Add 15 c.c. of the 0.02 per cent benzidine solution. Add 3 c.c. of the 0.25 per cent acetic acid. Add 1 c.c. of the 1 per cent hydrogen peroxide. Mix by inversion and place in a beaker of ice water containing plenty of ice. Allow to remain from thirty-five to forty minutes. Do not remove tube until ready to read. Place some of the solution in the left colorimeter cup and read the artificial standard in the right cup. It is important that the colorimeter cups and plungers are very clean. Wash with 10 per cent acetic acid, then distilled water and finally the solutions to be read.

## Calculation:

$X = \text{gm. Hb. per 100 c.c. of blood.}$

$S = \text{reading of standard.}$

When unknown is set at 10 mm.

$X = S.$

## EXPERIMENTAL

The usual experiments of varying the amounts and concentrations of the three principal reagents, benzidine, acetic acid, and hydrogen peroxide, were made. Too strong benzidine caused a precipitate to form while weaker solutions gave less intensity of color. If stronger acetic acid was used, the color intensity was less while with weaker acid the color was more unstable. One per cent hydrogen peroxide gave the maximum color. With both stronger and weaker solutions the color developed was less.

Bloods were prepared with concentrations of hemoglobin varying from 4 gm. to 20 gm. per 100 c.c. and the proportionality of color concentration was a straight line when read against the artificial standard.

It was found that the maximum color development was reached in about thirty-five minutes when it remained constant for about twenty minutes and then faded or became slightly purple.

The proper color concentration of the artificial standard was found by determining carefully the hemoglobin concentration of a sample of blood by its oxygen capacity  $\times 0.746$ . By varying the strength of the standard a color concentration was produced which exactly matched that of 10 gm. Hb. per 100 c.c. of blood when analyzed according to method.

\*The ordinary Hayems fluid cannot be used as diluent. An isotonic solution containing 0.65 per cent NaCl plus 0.15 per cent sodium oxalate is satisfactory.

The hemoglobin contents of twenty bloods were determined by their oxygen capacities and by the present method. The variation between the methods was not greater than 4 per cent.

Emphasis must be placed on the necessity of cleanliness and accuracy in preparing reagents. If conditions are carefully followed, it is believed that the use of the artificial standard will be very satisfactory for routine work.

#### SUMMARY

The blue color obtained when benzidine, acetic acid and hydrogen peroxide are added to hemoglobin has been standardized as a microhemoglobin method.

An artificial standard has been prepared for routine work.

The hemoglobin content of 1 c.mm. of blood may be determined.

The author wishes to extend his thanks to Dr. W. G. Karr for advice and suggestions during the progress of this work.

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## THE BACTERICIDAL POWER OF BLOOD\*

REUBEN OTTENBERG, M.D., NEW YORK, N. Y.

IN a recent publication Frederick W. Shaw<sup>1</sup> discusses the question of the influence of the bactericidal power of the blood on the technic of taking blood cultures. He shows from citations of the literature that immune serum is not bactericidal but offers a satisfactory culture medium for bacteria. From his own experience in which he used defibrinated blood of the patient himself as a culture medium, he believes that this is a satisfactory procedure and that the natural bactericidal property of the blood can safely be neglected.

I have no doubt that when there are sufficient microorganisms in the blood to survive the first hours of incubation when the bactericidal power of the blood is at its highest, positive blood cultures can often be obtained by this technic. However, experience with blood cultures, and in particular some experiments performed in 1931 indicate that when the numbers of organisms per cubic centimeter of blood is not large, the natural bactericidal power of the blood may very well interfere with obtaining positive results.

The experiments in question were not undertaken to answer directly the question discussed above. They were incidental control observations in the study of the bactericidal power of an antiseptic which it was thought might be used intravenously in bacteremias. As the study with the antiseptic was negative, the experiments were not published at the time. But the control observations necessitated the answering of certain definite questions, namely: 1. Is citration or defibrination preferable for experimental work? 2. Does citration impair the normal bactericidal power of the blood? 3. How bactericidal is normal blood for *Streptococcus viridans*? 4. For how long does the bactericidal effect of the blood continue? 5. How bactericidal is the patient's blood in subacute endocarditis for his own organisms and for organisms derived from other sources?

1. Is citration or defibrination preferable for experimental work (with bactericidal drugs)?

A *Streptococcus viridans* culture (No. 90) obtained from a case of bacterial endocarditis was used. It grew very rapidly, emulsifying well on shaking. Of a twenty-four-hour culture diluted 1:1,000, 1 c.c. was placed in each of two small flasks at the patient's bedside. One of the flasks contained a number of glass beads and the other 0.3 c.c. of 10 per cent sodium citrate. The patient had a case of polycythemia. Twenty cubic centimeters of blood were obtained from the patient and 10 c.c. put into each flask. The two flasks were then shaken

\*From the Laboratories of Mount Sinai Hospital, New York.

Received for publication, January 8, 1934.

†In the following report the word "bactericidal" is not used in the immunologic sense as for example in speaking of the Pfeiffer phenomenon, an amboceptor-complement reaction; it is used in the broad sense of a phenomenon which causes apparent disappearance of bacteria from the blood.

equally for ten minutes. Then 0.1 c.c. was removed from each flask, mixed in 5 c.c. of bouillon, and then plated with 10 c.c. of agar. After twenty-four hours the plate from the citrated blood showed 800 colonies, that from the defibrinated blood, 150 colonies. It was apparent that defibrination had removed a considerable number of bacteria, which were probably in the clot. For purposes of blood culture, particularly for immediate subcultures of blood on different media, and for the present experiments, citration would appear preferable to defibrination.

2. Does citration, as compared with defibrination, impair the normal bactericidal power of the blood? The technic was similar to that in the preceding experiment (blood was used from a patient with chronic cholecystitis) except that the bacteria were added to both flasks after instead of before defibrination. Dilution of the culture was higher, 1:20,000, in order to have a smaller number of colonies per cubic centimeter. Subcultures of 0.1 c.c. and 0.5 c.c. of blood from each flask were made at once and after the flasks had been in the thermostat one hour at 37° C. The results, read after forty-eight hours' incubation of the plates (in order to give all the colonies time to appear), are shown in Table I (the numbers indicate colonies per plate).

TABLE I

		SUBCULTURES AT ONCE	SUBCULTURES AT 1 HR.
Citrated blood	0.1 c.c.	32	21
	0.5 c.c.	137	35
Defibrinated blood	0.1 c.c.	42	12
	0.5 c.c.	126	51

The bactericidal effect is seen to be about as pronounced at one hour in the citrated as in the defibrinated blood. It is evident then that the addition of a small amount of sodium citrate does not impair the normal bactericidal power of blood.

3. How bactericidal is normal blood for *Streptococcus viridans*, and (4) how long does the bactericidal effect of blood continue?

This was determined on numerous occasions and showed very wide variation. In general it can be said that with different strains of *Streptococcus viridans* after one hour's incubation, about one-third to one-half of the organisms present disappear. After two hours' incubation about one-half to two-thirds or more disappear. After twenty-four hours in many instances 90 per cent or more of the microorganisms disappear from the blood. In other cases the bactericidal effect appears exhausted at this time and rapid multiplication of the bacteria ensues. In the following experiments 0.1 c.c. of a 1:15,000 dilution of a twenty-four-hour culture of a strain of *Streptococcus viridans* was added to 4 c.c. portions, respectively, of normal citrated blood and of bouillon. At once and again after two and one-half hours' incubation in the thermostat and after twenty-four hours at room temperature, subcultures of 0.1 c.c. each were plated and the colonies counted. The results are shown in Table II.

TABLE II

	COLONIES PER PLATE		
	SUBCULTURE AT ONCE	SUBCULTURE AT 2½ HR.	SUBCULTURE AT 24 HR.
Blood	512	52	6
Bouillon	480	692	Uncountable

Two similar experiments (both with normal blood, one with a hemolytic streptococcus) in which the primary bactericidal period was followed by rapid growth, are shown in Tables III and IV.

TABLE III

	HEMOLYTIC STREPTOCOCCUS		
	SUBCULTURE AT ONCE	SUBCULTURE AT 1 HR.	SUBCULTURE AT 24 HR.
Blood	115	14	Uncountable
Bouillon	80	100	Uncountable

TABLE IV

	STREPTOCOCCUS VIRIDANS		
	SUBCULTURE AT ONCE	SUBCULTURE AT 1 HR.	SUBCULTURE AT 24 HR.
Blood	125	65	Uncountable
Bouillon	65	100	Uncountable

5. How bactericidal is the patient's blood in subacute bacterial endocarditis for his own organisms and for organisms derived from other sources?

A rather high bactericidal power of the blood of cases of bacterial endocarditis for the homologous organism was observed in a number of experiments. When the number of organisms at the start was small, they were often killed off entirely in a few hours. Thus the following experiments were done with the blood of patients in the active stage of bacterial endocarditis.

To 5 c.e. portions of the patient's citrated blood were added 0.5 c.e., respectively, (a) of a heterologous *Streptococcus viridans* (strain No. 90), (b) of the patient's own *Streptococcus viridans* (strain No. 70), and (c) of sterile bouillon as control. At once, again after one hour, and after three hours, 0.5 c.e. of blood was plated and the colonies counted. By chance the number of organisms of strain No. 90 was too large at the start so that no count could be made, but it was obvious from the three-hour plate that this strain had multiplied vigorously. On the other hand, with the patient's own strain the large number of added organisms was greatly reduced at three hours, and in the sample of the patient's blood to which no further organisms were added the small number originally present (11 per 0.5 c.e.) were completely killed off.

TABLE V

	SUBCULTURE AT ONCE	SUBCULTURE AT 1 HR.	SUBCULTURE AT 3 HR.
(a) Blood plus Culture No. 90 (heterologous)	Uncountable	Uncountable	Uncountable but increased
(b) Blood plus Culture No. 70 (homologous)	50,000 (approximate)	3200 (approximate)	1000
(c) Blood without added bacteria	11	2	0

In the following experiment the bactericidal effects of a normal blood and of the blood of a patient suffering from bacterial endocarditis were compared on three strains of *Streptococcus viridans*, one of which (No. 14) was derived from the patient herself. To 4 c.c. portions of citrated blood was added 0.1 c.c. of a 1:20,000 dilution of a twenty-four-hour bouillon culture of each organism; 0.5 c.c. amounts of blood were plated.

TABLE VI

	CULTURE ADDED	SUBCULTURE AT ONCE	SUBCULTURE AT 2 HR.
Normal blood	No. 90	Uncountable	Uncountable but in- creased
	No. 70	2400	800
	No. 14	3400	1600
Patient's blood	No. 90 (heter)	2200	1200
	No. 70 (heter)	2400	500
	No. 14 (auto)	3200	220
	No addition of bacteria	250	85

It is seen that the patient's bactericidal power for the heterologous strain (No. 70) is of about the same order as that of normal blood. The initial growth of strain No. 90, another heterologous strain, in the patient's blood seems to have been inhibited as compared with its growth in normal blood; here it appears as though the inhibitory effect of the immune blood on a heterologous strain manifested itself in the poured plate. The patient's blood evidently reduced the number of her own added organisms (strain No. 14) at two hours very much more than did the normal blood. It appears that the inhibitory power of normal blood can be overwhelmed if the initial number of organisms is too great. Strain No. 90 had previously been shown to be quite susceptible to the normal bactericidal power of the blood when not initially present in excessive numbers; it was the strain used in most of the preceding experiments.

## SUMMARY

1. Defibrination removes large numbers of bacteria from the fluid portion of the blood. They are probably held in the clot.
2. Sodium citrate does not impair the normal bactericidal power of the blood.
3. On incubation of the blood for a few hours, bacteria (*Streptococcus viridans* and hemolytic streptococcus) disappear in large numbers from normal blood, and in even larger numbers from the blood of patients infected with the homologous strain. The question whether this phenomenon is due to antibodies, phagocytosis, or disintegration of leucocytes with liberation of bactericidal substances, is not discussed.
4. The determination of bactericidal effects depends largely on the initial number of bacteria present. When this is very small, the bacteria may be completely killed off. When it is very large, the bactericidal effect may be completely lost.

## CONCLUSIONS

The above experiments indicate that the normal and the immune bactericidal power of the whole blood cannot safely be disregarded and that the factor of



dilution probably plays an important rôle in the results obtained in blood cultures. They also show how carefully any experiments on chemotherapy of bacterial infections of blood need to be controlled.

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## A METHOD FOR QUANTITATING THE REDUCING SUBSTANCE OF THE ANTERIOR HYPOPHYSIS\*

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WILLIAM T. SALTER, M.D., ARDA GREEN, M.D., AND TRACY J. PUTNAM, M.D.,  
BOSTON, MASS.

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CHROMOPHILE adenomas of the hypophysis and aqueous extracts of the anterior lobes of animals contain a substance which actively reduces methylene blue, and which is not found in chromophobe adenomas or in extracts of other organs.<sup>1</sup> Hemoglobin does, of course, reduce methylene blue, but the reduction produced by anterior hypophysis substance occurs in the absence of hemoglobin. The present investigation was intended to study the chemistry of this reaction further and to determine which chemical fraction of the crude anterior lobe extract contained the reducing substance. A mildly alkaline extract made according to Evans' original formula,<sup>2</sup> which had a high reducing activity, was used as a starting point.

The method used for studying the activity of various preparations was based upon Ahlgren's technic.<sup>3</sup> The procedure consists in determining the time required to bleach a standard amount of methylene blue contained in an evacuated Thunberg tube under arbitrarily selected conditions.

*Effect of Acidity.*—It was found that between  $P_H$  10 and  $P_H$  8, little change in reducing time occurred; but at more acid reactions, the reducing effect fell off very rapidly. Below  $P_H$  6, reduction was negligible. Accordingly, our test solutions were buffered at  $P_H$  8 with Sorensen phosphate buffer, the final concentration of which was made up to about 0.06 molar.

*Effect of Concentration of Methylene Blue.*—At constant temperature and constant acidity, the time required by a known concentration of pituitary extract to effect complete bleaching was a direct linear function of the methylene blue concentration. This relationship failed, however, when such excessive amounts of dye were used that the reducing capacity of the pituitary substance was exceeded.

*Electrolyte Concentration.*—This had no appreciable effect within wide limits at the temperature and acidity employed.

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\*From the laboratories of the Collis P. Huntington Memorial Hospital, Boston, and the Department of Neuropathology, Harvard Medical School.  
Received for publication, December 8, 1933.

*Effect of Temperature.*—The effect of temperatures below 50° C. is uncertain because of the possibility of bacterial contamination which in some experiments gave an apparent optimum close to 40° C. Above 60° C., however, bleaching proceeded with increasing rapidity as higher temperatures were employed. Thus in one experiment the bleaching time at 80° C. was treble that at 60° C. In practice, it was found that reducing time could conveniently be read at 100° C. in solutions which failed to bleach completely at 40° C. even after forty-eight hours. Accordingly, bleaching activity was measured regularly at 100° C. by immersing the vacuum tubes in a bath of gently boiling water.

It should be emphasized that this method is a dynamic one, based upon rate of reaction. It was found that comparable results could be obtained by a titration method in which the amount of dye to be reduced was varied. This procedure, however, proved much more tedious and less accurate than the one employed.

*Detailed Technique.*—Into each Thimberg tube was placed 0.1 c.c. of 1:1000 methylene blue and 0.15 c.c. of molar phosphate buffer at P<sub>H</sub> 8.0. Then the solution to be tested (adjusted to P<sub>H</sub> 8.0) was added, and the total volume made up to 2.5 c.c. with distilled water. The tube was then evacuated as described by Ahlgren by means of a Cenco vacuum oil pump protected by a trap. Evacuation was begun cautiously until the initial frothing had subsided, then continued until (after about three minutes) brisk boiling had subsided and the tube had become perceptibly chilled (to the hand). It was then placed in boiling water and the time noted. Observations were made every half minute until complete bleaching was noted by comparing with a previously bleached control.

*Standard Bleaching Curve.*—A sterile alkaline extract of anterior pituitary substance,\* made according to a modification of Evans' method, was arbitrarily selected as standard. The total volume was 2.5 c.c. The respective bleaching times for successive volumes of gland extract is shown in Table I.

TABLE I

VOLUME IN 2.5 C.C.	BLEACHING TIME IN MIN.
0.2 c.c.	45
0.3 c.c.	30
0.4 c.c.	19
0.5 c.c.	15
0.6 c.c.	11
0.7 c.c.	9
0.8 c.c.	6
1.0 c.c.	6
1.2 c.c.	4
1.4 c.c.	4
1.6 c.c.	3

*Unit of Activity of Gland Extract.*—An "alpha unit" was arbitrarily selected to serve as a measure of the apparent potency of various extracts. It was arbitrarily decided that an amount of extract containing 10α would

\*Kindly provided by Parke, Davis & Company.

reduce the standard amount of methylene blue in fifteen minutes. Final dilutions in each case were adjusted so that the reducing time would approach fifteen minutes.

*Extracts Tested.*—To begin with, various commercial extracts of the posterior lobe and preparations from pregnancy urine containing an estrogenic substance supposedly of hypophyseal origin were tested and found to have no effect upon methylene blue.

Next, various globulin fractions of the crude alkaline extract were tested, as it is well known that the growth-stimulating hormone tends to remain with the globulin fraction. It was found that any chemical manipulation tends to decrease the reducing activity of a given extract. Since hemoglobin reduces methylene blue, the crude extract was brought to  $P_H$  5.0, the hemoglobin filtered off, and the globulin precipitate redissolved at  $P_H$  7.5. To give a concrete instance, one sample of crude extract contained 67a units and 6.3 mg. nitrogen per c.c. The filtrate (containing hemoglobin) showed 16a units and 0.5 mg. nitrogen per c.c. The precipitate, redissolved and made up to volume, contained 12a units and 4.2 mg. nitrogen per c.c. This solution was again precipitated in a similar manner and the precipitate redissolved and made up to volume when it showed 11a units and 3.3 mg. nitrogen per c.c. Such redissolved precipitates often displayed considerable growth-promoting activity; a completely hypophysectomized rat injected daily with 1 c.c. of one extract gained 28 gm. in twelve days. But the results were inconstant. The filtrates never showed any growth-promoting potency.

Further fractioning of the globulin precipitate (by salting out or dilution) yielded contradictory results. Both the a value and the growth-promoting qualities were diminished markedly in both filtrates and precipitates.

Finally, Professor J. B. Collip<sup>4, 5, 6, 7, 8</sup> of Toronto most kindly furnished us with samples of fractions, each of which has a distinct physiologic activity. The growth hormone, which Professor Collip informs us produces active growth in hypophysectomized rats when given in doses of  $\frac{1}{64}$  c.c. twice daily, contained no reducing substance. The thyrotropic fraction contained none. The adren-alotropic fraction contained a trace. The maturity hormone contained 1.3a units per cubic centimeter—certainly a very small amount.

#### SUMMARY

1. A method is described for quantitating the reducing power of the tissue of the anterior lobe of the hypophysis.
2. Active reduction may be produced by extracts devoid of hemoglobin.
3. The reducing substance tends to remain with the globulin fraction, which usually also contains the growth-promoting hormone, but the two do not appear to run parallel.
4. Extracts containing the growth, thyrotropic, adrenaltropic, and maturity hormones, respectively, supplied by Dr. Collip, contained no more than traces of reducing substance.

NOTE: After this article was ready for press, our attention was called to an article by Gough and Zilva<sup>8</sup> also describing a reducing substance in the anterior pituitary, which these authors identify with ascorbic acid.

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## IMPROVED METHODS IN THE CULTURE OF STERILE MAGGOTS FOR SURGICAL USE\*

WILLIAM ROBINSON, PH.D., WASHINGTON, D.C.

THE use of sterile blowfly maggots in the treatment of infected wounds, introduced to surgery by Baer,<sup>1</sup> has grown rapidly during the last two years, and the maggot treatment has now been given in every state in the United States and in several foreign countries. Maggots are usually purchased from certain medical supply houses, but an increasing number of hospitals are now producing their own supply. With the proper equipment and training the private rearing of maggots is quite practicable. The Bureau of Entomology has developed methods by which sterile maggots can be produced on a small scale both conveniently and economically. Some knowledge of the biology of blowflies, as well as familiarity with rearing methods and bacteriologic technique, is essential. Since the former article<sup>2</sup> was written the improvements in cultural technique have been so extensive as to require a revision of the whole process.

### SPECIES OF MAGGOTS SAFE TO USE

Both *Lucilia sericata* and *Phormia regina* are satisfactory for cultural purposes and also for use in wounds. *Calliphora erythrocephala* has been found to give less favorable results in the wound and is more difficult to rear throughout the year. Its use was reported in one large series of cases in the United States, but specimens later sent into the Bureau of Entomology for determination were identified as *L. sericata*. These species are all safe for surgical use. There

\*From the Division of Insects Affecting Man and Animals, United States Bureau of Entomology.

Received for publication, March 10, 1934.

is serious danger, however, in the use of screw worms, which belong to the genus *Cochliomyia*, and they must be carefully avoided as they do not distinguish between dead and living tissues and will penetrate healthy tissue.

#### BIOLOGY OF FLIES AND MAGGOTS

The eggs, which develop into maggots, are laid by the flies upon meat in colony cages. They are small and whitish and are usually laid in masses. As the eggs are soft, they should be handled carefully. Hatching occurs within twenty-four hours. Eggs are used for two purposes: (1) for propagation of new generations, as flies live only from four to six weeks; (2) for production of sterile maggots to be used in wounds. The eggs for both these purposes are obtained from a common source and no distinction is made until later. Treatment of eggs for propagation is discussed in the next section, and for surgical use in the following one.

Maggots begin to feed shortly after hatching. They partially liquefy their food and take it up in this condition. They are pointed at their head end. When feeding in liquids, they breathe through two spiracles in the heavy, posterior end of the body. Maggots have no eyes, and light is not necessary for their development. Those intended for propagation, called brood maggots, grow rapidly and become full size in from five to seven days. They then leave their food in search of a secluded place to pupate.

After the maggots have migrated, but before pupating, they remain in a prepupal, maggotlike, stage for several hours. In this stage further development is sometimes retarded by placing the specimens in cold storage at from 40° to 45° F. Although this retardation is a convenience, the practice is inadvisable as the Bureau of Entomology<sup>2</sup> has found it to destroy a large percentage of the specimens.

With continued development the prepupa changes into a brownish, oval pupa. This is a quiescent stage, and no feeding takes place. Transformation from the maggot to the fly occurs in this stage and occupies about seven days. Maggots that have been used surgically do not pupate as they are destroyed after removal from the wound.

The female fly normally begins laying eggs from five to ten days after its emergence. The number laid daily varies considerably but averages about 100. The egg-laying period covers from four to six weeks, but the egg-laying capacity of flies and the viability of the eggs begin to decline in three weeks, after which the old colonies should be destroyed and replaced with fresh young ones. Continued inbreeding of the same strain is apparently not detrimental to the progeny.

During autumn and early winter some irregularity in egg-laying may occur. For some days very few eggs may be laid, and it may be supposed that the colonies are degenerating. Resumption of egg-laying, however, usually takes place within a few days. These fluctuations which occur although summer conditions are supplied in the fly cabinet, are regarded as the result of an inherited tendency to cease activity in winter.

While any range of temperature between 70° and 85° F. is tolerated throughout the life cycle, 80° is especially favorable. A uniform temperature is advisable to control the rate of growth and activity. A moderate degree of humidity in the air is desirable in all stages of development, and 50 per cent gives good results. Under these conditions the life cycle of the species is from forty-five to sixty days. A method of supplying heat and humidity is described below.

#### MAINTENANCE OF REARING STOCK

*Incubator or Rearing Cabinet.*—As blowflies normally hibernate in winter, the laboratory production of maggots at this time is unnatural. Accordingly additional heat and humidity are supplied in winter to keep the species active. A cabinet for this purpose can be made at small cost. It is constructed of wood with glass doors and with a shelf that allows two layers of cages to be used. The size will, of course, depend upon the number of colony cages used. Inside dimensions of 4 feet in height, 4 feet in width, and 2 feet in depth will be ample for 12 cages.

Electric light bulbs installed on the floor of the cabinet at one end and connected with a thermostat will supply heat conveniently. About six 100-watt bulbs would ordinarily be sufficient. This number will use 6 amperes of current, so a thermostat of sufficient capacity should be used, otherwise a relay should be added. A switch which permits three heats is also advisable.

The humidifier, placed at the other end of the cabinet, consists of a shallow, broad pan of water with a frame to allow lengths of absorbent cloth to hang in the water. A baffle board or false floor, having a number of one-inch holes, is supported above the heater-humidifier to prevent most of the air from rising directly into the cabinet but allowing it to rise mainly around the edges of the board. The part of the floor above the heater is protected by a sheet of asbestos. An opening 4 inches square covered with wire gauze is made in the side of the cabinet by the heater to allow fresh air to enter, and from 4 to 6 one-inch holes are made near the top of both sides for exit. A small electric fan, placed between the heater and the water pan to force the air forward, will give more general circulation and is recommended. The actual degree of humidity in the air is difficult to determine, and most private laboratories will probably have no record of the humidity. The humidifier should then be used continuously, and in winter several thick absorbent cloths should be suspended in the water. With an electric fan fewer cloths are needed. The ordinary dry air of the laboratory in winter is detrimental to development.

In rearing maggots for propagation, when raw meat is used, disagreeable odors will develop. A smaller separate cabinet is advisable, therefore, with an exhaust fan on an outside vent to draw off the odors at the top of the cabinet. Unpleasant odors do not occur in the culture of sterile maggots.

*Cages for Fly Colonies.*—Flies are kept in colonies, each consisting of about 150 individuals. A simple cage can be made of gauze over a frame of wood, from 10 to 12 inches in each dimension. Upright posts are secured

firmly to the corners of the solid wooden floor and supported at the top with horizontal pieces on all four sides. This is covered on the sides with muslin gauze held in place with thumb tacks. In one of the sides a sleeve of the same material is attached for access to the cage. The top is covered with metal screening so the inside will be visible. This is an inexpensive cage and is easily made.

Another type devised by Simmons<sup>7</sup> is especially rugged and serviceable and permits rapid handling and cleaning. It is more expensive, but it will outlast the other cage. The top has a large circular opening and is braced beneath to prevent cracking. The sides are six metal rods threaded at top and bottom and held in place with two nuts at each end. The cage is 14 inches high and 10 inches in diameter. A cloth sack of muslin lawn is made to be pulled up over the cage and fastened at top and bottom with a cord in a slot cut around the edges. The sack has a short sleeve as described for the other cage, and the top is covered with metal screening, celluloid, or cellophane.

*Care of Fly Colonies.*—No consideration need be given to the proportion of the sexes. The ratio is usually 1 to 1, and the number of males that occur in the colonies is satisfactory for fertilization.

The food of flies differs greatly from that of maggots. Flies will thrive if a Petri dish of granulated sugar is kept in each cage and supplemented twice a week with Baer's mixture<sup>1</sup> of 70 c.c. water, 30 c.c. honey, one quarter cake of yeast, and one egg. This mixture is best supplied on a pad of absorbent cotton in a separate dish. The remainder will stay fresh only a few days and should be stored in the refrigerator. Flies require water, and this is conveniently supplied by a water fountain described by Miller.<sup>2</sup> It consists of a small beaker of water inverted upon a Petri dish containing one or two sheets of filter paper in the bottom. Flies would drown in water supplied in an open dish.

A piece of lean beef is placed in another dish for the flies to lay eggs upon. For a colony of 150 flies a piece of meat two inches square is ordinarily sufficient. Under the "one-day" method of collecting and sterilizing eggs, described later, a larger piece is used. The meat is given as soon as the flies emerge as it stimulates egg-laying.

No special care is required to provide light for the flies. The ordinary light of the laboratory is sufficient. Exposure of the colonies to direct sunlight or ultraviolet light is unnecessary. Flies are attracted to a bright light, and when cages are opened a light shining through the opposite side will draw them and prevent their escape.

Cages should be kept reasonably clean. While sterility of surgical maggots does not depend upon aseptic conditions in the fly cages, it is advisable to avoid unnecessary contamination of the eggs. When cages become noticeably soiled, flies can be transferred by inverting a clean cage over the soiled one and removing the intervening screen. Transference is hastened by darkening the lower cage.

*How to Avoid Dwarfed Flies.*—The only function of flies is the production of eggs, and for maximum usefulness flies should be large and vigorous.

Dwarfed flies lay fewer eggs, and their life is much shorter. Several factors can cause dwarfing of flies. The injury is done in the maggot stage; flies cannot change in size. If maggots are forced to migrate from their food prematurely, it is almost impossible to get them to resume feeding after correcting the trouble. They will tend to pupate and eventually emerge as small flies. Premature migration can be caused by: (1) insufficient amount of food for brood maggots; (2) placing excessive numbers of eggs upon the meat and causing overcrowding of maggots; (3) accumulation of gases in the feeding cage, through insufficient ventilation. The fourth cause of dwarfed flies is prolonged cold storage of prepupae. A loss of water which takes place in storage results in both high mortality and stunted flies. Instructions for the proper management of feeding are given in a following section.

*Cage for Brood Larvae.*—To replace fly colonies before egg production begins to decline, a certain proportion of maggots must be reared through to the fly stage. Cages can be made of glass beakers, dressing jars, or commercial oiled-fiber cans.<sup>4</sup> The latter has several advantages over the others. The cage consists of two containers; an inner one about 3 inches wide to hold the meat, and an outer one from 2 to 3 inches wider and higher for pupation. An inch of sand is placed on the bottom of the outer container. To exclude parasites or other invaders, it is well to cover the outer cage with fine gauze. A heavier material should be avoided as some ventilation is necessary to prevent accumulation of injurious gases.

*Care of Brood Larvae.*—A larval cage of these dimensions is large enough for the development of about from 250 to 300 maggots. The inner container is three-quarters filled with raw, lean beef such as scraps, hamburger, or other inexpensive pieces. The meat should be moist. Plenty of food must be provided to avoid premature migration as already discussed. Approximately from 200 to 300 eggs, weighing from 20 to 30 mg. are placed down among the meat, and the inner container is left uncovered and put on top of the sand in the outer one. Under favorable conditions the maggots will feed until full grown and then migrate to the sand to pupate. After migration the food residue, which is foul-smelling, should be disposed of at once. Occasionally a few maggots fail to migrate, and they should be discarded with the residue. After migration the larvae can be left in the sand until they pupate, or placed in a beaker with sand in clean colony cages.

*Removal of Odors.*—Foul odors which develop during production of brood larvae need not become objectionable. The ventilator described for the larval cabinet will remove odors effectively. A household fan, however, used within the cabinet to force air up the pipe is inadequate; and a small exhaust fan in the ventilator line to draw off the odors is practically essential. Residue from brood cages and other waste can be kept conveniently in a paper bag within a large dressing jar or fiber can. This should be stored in the larval cabinet or a chemical hood and burned each day.

*First Steps in Maggot Production.*—Vigorous colonies of flies are one of the first requisites. Foundation stock to start colonies can usually be obtained from a laboratory already producing maggots. It is best shipped in the



prepupal or the early pupal stages and enough should be requested to start three colonies. Upon arrival from 100 to 150 should be placed at once in each fly cage. If any have not yet pupated, a beaker with an inch of sand should be used. Granulated sugar and the water fountain are added and the cages placed in the rearing cabinet. When adults appear, the meat should be included and renewed often enough to keep it from drying. Flies will begin to lay eggs in from five to ten days.

A large number of maggots should be reserved for the next generation of flies before the production of sterile maggots is attempted. As to the number of colonies to maintain, a convenient index is that each female will lay a daily average of about 100 eggs. A colony of 150 flies will, of course, have only about 75 females.

#### CULTURE OF STERILE MAGGOTS

The same colonies of flies that produce brood larvae are also used in the culture of surgical maggots. Maggots for use in wounds are cultured under aseptic conditions, and careful bacteriologic technic must be employed. The maggots cannot be sterilized, but sterility can be obtained in the egg stage and the maggots kept from contamination by the use of sterile containers and food.

*Removal and Care of Eggs.*—The eggs are laid in masses upon the meat, usually in crevices or around the lower margins. Because of their short hatching period all eggs are removed with meat from the fly cages at the end of the day. Egg masses are carefully picked off with a scalpel and placed on a piece of wet cloth laid on a wet cotton pad in a Petri dish. If clumps are large it is best to place them on half the cloth, turning the other half over them. The wet cloth facilitates separation of the eggs described in the next section. The dish is covered and stored in the refrigerator until the next morning when the eggs are separated and sterilized.

*Separation of Eggs.*—This is necessary before sterilization so the disinfectant can reach the entire surface of each egg. Incomplete separation may result in failure to sterilize. The most satisfactory method tried is the one devised by Simmons.<sup>8</sup> The process includes the moistening of the eggs on the wet cloth already described. This is done to soften the mucoid material around the eggs. In thirty minutes' contact with the wet cloth the eggs can be separated readily by spreading them thinly on the cloth with a spatula. Black cloth is advisable as small clumps needing further separation can be seen. The eggs are then ready for transference to the disinfectant.

*Sterilization of Eggs.*—Formalin compares favorably with the numerous other disinfectants tested, and in some respects it is the most satisfactory one tried. Immersion of the eggs in a 5 per cent solution of formalin plus 1 per cent sodium hydroxide for five minutes has been found sufficient to produce sterility in routine maggot culture. The sodium hydroxide was added by Simmons to prevent agglutination of the eggs in the disinfectant, a tendency existing in eggs separated by the wet-cloth method.

Eggs are best sterilized in small lots. For private use, from 500 to 600 is a convenient number for a lot and usually as many as needed in a wound; overcrowding the wound frequently leading to trouble.<sup>6</sup> When produced commercially for shipment the number is better increased to 1,000. These small lots are more adaptable to aseptic technique. The number of eggs in a mass can be determined easily by the weight method,<sup>4</sup> approximately 10 eggs (unmoistened) weighing 1 mg. After a few lots have been weighed the number can be estimated by its size.

A test tube of disinfectant is used for each lot of eggs. The separated eggs are lifted from the wet cloth with a spatula or scalpel, placed in the disinfectant, and stirred gently. After five minutes most of the solution is decanted and the eggs are then washed. A convenient apparatus<sup>6</sup> for washing the eggs consists of a Gooch crucible containing a piece of bandage gauze about 2 in. square and supported in the neck of a wide-mouth specimen bottle. The eggs are poured from the test tube on to the gauze and washed with 100 c.c. of water. A separate unit of this kind is used for each lot of eggs. The crucible and gauze are protected from contamination by covering them with an inverted crystallizing dish which rests upon the shoulders of the bottle, and the unit is, of course, previously sterilized intact. The eggs are transferred upon the gauze directly to the food.

*Sterile Maggot Food.*—The maggots are reared in glass containers. Shell vials 85 mm. high and 35 mm. wide, or 2-ounce specimen bottles with extra wide mouth and small shoulders, make convenient containers. They are cleaned and plugged with gauze-covered cotton and sterilized. The food is introduced next.

There are now two types of sterile food. The older type makes the maggots grow rapidly and consequently retardation of their growth in the refrigerator is necessary during the sterility tests (described in the next section). The chief objection to this method is that cold storage kills a large percentage of the maggots.<sup>5</sup> Several formulas are in the literature.

The newer type of food permits only a slow rate of growth up to the time of implantation but does not interfere with feeding and rapid growth in the wound. This food, recently devised by Simmons,<sup>9</sup> consists of one part of evaporated milk to from five to seven parts of water, to which is added 1.5 per cent plain agar. The mixture is cooked in a double boiler for 20 minutes, and about 15 c.c. is poured into each container. The advantages of this retarding food are that it eliminates cold storage of maggots during the sterility tests, and it permits holding of surplus maggots in reserve a few days without chilling and consequent mortality. The food is also cheap and easy to prepare; and it permits shipment of sterile maggots long distances without the customary ice packing.

After the food is added the containers are replugged, autoclaved for thirty minutes at 15 pounds pressure, and stored in the refrigerator. When eggs are introduced, the bottles are kept in the fly cabinet for hatching. The cloth is best left in the bottle until implantation.

<sup>6</sup>Devised by Dr. G. F. White, U. S. Bureau of Entomology.

*Test for Sterility of Maggots.*—About forty-eight hours after hatching, each lot is tested for sterility. A good method is to test some of the partly liquefied food upon which the maggots are feeding. Both aerobic and anaerobic tests must be made. If the retarding food is used, the maggots can be left at room or incubator temperatures while the bacteriologic cultures are being incubated; otherwise their growth must be restrained in the refrigerator. Any lots found contaminated forty-eight hours later must be discarded. The remainder are suitable for surgical use. By this time the maggots are usually from 4 to 6 mm. in length, a size best suited for use in the wound. Larger maggots are less desirable as their feeding period in the wound is too short. Negative cultures are incubated from four to five days longer, as an extra precaution, after the maggots have been released for clinical use. With some practice in the sterilizing technique, a high percentage of sterile lots should be obtained.

*Cold Storage of Surgical Maggots.*—When an excess of sterile maggots occurs, a common practice has been to store the surplus in the refrigerator until needed. As this causes a high mortality, it is better when a non-retarding food is used to restrict the supply rather than to depend upon cold storage. If the retarding food is used, cold storage is unnecessary and surplus maggots can be held at room temperature for a few days.

#### A ONE-DAY METHOD OF OBTAINING AND STERILIZING EGGS

A cultural method has been devised<sup>6</sup> whereby eggs may be sterilized the day they are laid. This is done upon alternate days and leaves each intervening day free for other work. The use of only young flies (up to three weeks of age) is especially advisable. Meat is given upon alternate days and larger pieces than usual (3 in. square) are used. Under these circumstances, flies have laid as many eggs in three hours as ordinarily during a whole day. The meat is placed in the fly cages about 9 A.M. and removed at noon. The eggs are then collected and placed upon the wet cloth. In the early afternoon the eggs are separated, sterilized, and transferred to the food as described under the longer method. As these eggs are newly laid and therefore easily killed during sterilization, it is important to use a disinfectant which will not injure them. The formalin solution as described is especially suitable. The food containers with eggs are then placed in the incubator.

#### PROBLEMS IN THE USE OF MAGGOTS IN WOUNDS

In the use of living maggots in human wounds, there arise certain problems of a biologic as well as a clinical nature. Some of these questions have been discussed from the biologic standpoint in a circular issued by the Bureau of Entomology.<sup>6</sup> The circular deals with such matters as the inability of maggots to reach deep necrotic areas without surgical assistance; the unfavorable effect upon maggots of accumulated wound secretions; implantation of maggots in the wound; avoidance of overcrowding of maggots; a description of various types of maggot retainers over the wound; why maggots sometimes die in the wound; and why they sometimes escape from the wound.

## SUMMARY

The use of sterile blowfly maggots in surgery has become widespread in the last three years, and the private culture of maggots is now being attempted in many hospitals. With the proper equipment and training the production of maggots for private use is quite practicable. Detailed descriptions are given of methods and equipment that have been found satisfactory for this purpose. An outline of the biology of blowflies as involved in the cultural processes is also given.

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# A MICROMODIFICATION OF THE FOLIN-WU BLOOD SUGAR METHOD USING PERMANENT STANDARDS<sup>c</sup>

HERMAN BROWN, B.S., PHILADELPHIA, PA.

THE Folin and Wu<sup>1</sup> blood sugar method has been adapted by various workers<sup>2, 3, 4</sup> to the determination of reducing sugar in minute amounts of blood. Such "micro" modifications have proved reasonably accurate for clinical analysis. There is also the added advantage over the more cumbersome ferricyanide method<sup>5</sup> in that a new set of reagents need not be prepared in shifting from the macro- to the micromethod, since as a rule the latter is used only intermittently in cases in which it is undesirable or difficult to obtain larger amounts of blood. Weaker sugar standards are usually required for the micromodifications and their intermittent use increases the uncertainty inherent in the relatively unstable sugar standards. It would, therefore, be desirable to have at hand permanent colored standards corresponding to definite amounts of sugar when such a modification is employed. These standards would also make the method useful clinically when a colorimeter is not available.

The above considerations led to an attempt to devise permanent standards which would not only be reasonably stable (stability over a period of one year was set up as a minimum requirement) but would also cover a range sufficiently wide to enable one to obtain results without the necessity of repeating the test. Standard colors that apparently fulfill these conditions have been prepared as follows:

*Solution A.*—Fifty milligrams of Alizarine Blue G.S.† and 10 mg. of Alizarine Blue-Black B.G.† are transferred to a 100 c.c. volumetric flask dissolved in distilled water and diluted to the mark.

*Solution B.*—A distilled water solution of 20 mg. of Alizarine Cyanone Green B extra† per liter.

From these solutions standards are prepared by measuring into test tubes of uniform bore amounts of each as given in Table I.

TABLE I

SUGAR MG. PER 100 C.C.	SOLUTION A C.C.	SOLUTION B C.C.
60	0.30	9.70
100	0.60	9.40
200	1.40	8.60
300	2.30	7.70
400	3.70	6.30
500	5.90	4.10

\*From the Research Institute of Cutaneous Medicine of Philadelphia.  
Received for publication, February 15, 1934.

†From the National Aniline and Chemical Company. It may be found that other dyes will give the same color values, however these were selected because they belong to the anthraquinone series and are among the fastest dyes known.

The values in Table I can be plotted on coordinate paper using values for sugar concentration and solution A as abscissa and ordinate, respectively. From the resulting curve such intermediate values for sugar concentration as may be desired can be picked off and standards prepared accordingly.

Below 200 mg. per 100 c.c. color values spaced 20 mg. apart are readily distinguishable permitting comparison with less than a 10 mg. error. Between 200 and 300 mg. standards 25 mg. apart have been found convenient and above 300 mg., 50 mg. spacings which permit interpolations of 25 mg. are sufficiently close.

These solutions, if placed in tubes (14 mm. bore has been found convenient) of noncorrosive glass which are hermetically sealed, will keep for at least ten months. The range covered will take care of practically all blood sugar values usually encountered if used in conjunction with the modification described below.

#### METHOD

*Reagents.*—1. Tungstic Acid Solution: To 40 c.c. of 10 per cent sodium tungstate in a liter flask add about 700 c.c. of water, then with shaking, 40 c.c. of 2/3 N sulphuric acid. Dilute to the mark and mix. A precipitate begins to form in this solution after about two weeks; however, it can still be used as a protein precipitant for about two or three months.

2. Alkaline Copper Tartrate: The original Folin and Wu<sup>1</sup> reagent is used. The less alkaline solution advocated later by Folin<sup>6</sup> is not only more troublesome but, as has been shown by Shaffer and Somogyi,<sup>7</sup> a much more alkaline solution must be used to obtain good recoveries in very dilute sugar solutions.

3. Color Reagent: The complex acid molybdate solution of Folin<sup>6</sup> is used. This solution not only gives a better proportionality of color to sugar content than the older reagent, but the color is considerably stronger for a given amount of sugar which is of distinct advantage when but small amounts of blood are used.

*Procedure.*—Five cubic centimeters of tungstic acid are placed in a test tube. With a 0.1 c.c. serologic pipette, blood obtained from an ear or finger prick (oxalated blood obtained by vein puncture may of course be used) is added to the solution, rinsing the pipette by drawing the tungstic acid solution into it several times. Stopper with the thumb and shake. The precipitated proteins may now be thrown down in the centrifuge or filtered. If filtered, a 7 cm. filter will be found sufficiently large to take all the mixture and give a filtrate of more than 3 c.c. Two cubic centimeters of this filtrate are placed in a test tube having a bore equal to that of the standard tubes, 2 c.c. of alkaline copper tartrate added, the tube stoppered with cotton and placed in vigorously boiling water for eight minutes. The tube is then cooled for about one minute without shaking, 2 c.c. of the acid molybdate reagent added and the contents mixed by side-to-side shaking. After frothing has ceased (about one minute) the color is compared with the permanent standard colors, giving the sugar content directly without calculation.

If the blood sugar is greater than 500 mg. per 100 c.c., sufficient filtrate is available to repeat the determination upon 1 c.c. plus 1 c.c. of water, the result after comparison of course being multiplied by two.

Comparison of colors is greatly facilitated by the use of a 3-hole wooden block, using either daylight or artificial light transmitted through a frosted glass plate.\* Such a block is readily prepared and its use makes color comparison sufficiently easy so that one may readily interpolate between standards.

#### COMMENT

The above modification of the Folin-Wu blood sugar method presents several advantages which should make it of value clinically: Neither marked tubes nor special tubes are necessary since no dilution of the final colored solution is required and the danger of partial reoxidation of the reduced copper oxide is avoided by stoppering the tube during boiling. The latter expedient has been successfully employed by many workers. Only one micro-measurement is made, that of measuring the blood sample, thus ensuring greater accuracy in the determination and avoiding the use of special pipettes. If the blood is collected outside the laboratory it may be transported in the tube in which the proteins have been precipitated without danger of loss of fermentable substances.

It will be noted that the time of heating has been increased from the usual six minutes to eight minutes. This has been done because it has been found<sup>†</sup> that the longer period is necessary for maximum reduction when dealing with very small amounts of sugar. A ten-minute heating period has been advocated by Shaffer and Somogyi but since their figures indicate that maximum reduction is approached at about eight minutes and to reduce the possibility of sugar destruction by the alkali, the latter figure has been adopted. That

TABLE II  
COMPARISON OF FOLIN-WU AND AUTHOR'S MICROMODIFICATION USING PERMANENT COLOR STANDARDS

SAMPLE	HUMAN BLOOD SUGAR—MG. PER 100 C.C.	
	FOLIN-WU	AUTHOR'S MODIFICATION
1	56	60
2	64	60
3	72	70
4	79	80
5	84	80
6	88	90
7	92	90
8	113	110
9	140	150
10	188	175
11	193	200
12	206	200
13	310	300
14	326	325
15	330	325
16	360	350
17	365	375
18	404	400

\*The complete equipment including sixteen colored standards may be obtained from the Standard Reagents Company, Philadelphia.

consistent results over a wide range of sugar values can be obtained by this modification is indicated by inspection of Table II which compares the results obtained on the same blood specimen by both the Folin and Wu and this micromodification.

Test tube color comparison is of course not as accurate as good mechanical colorimetry, nevertheless results by either method are probably more accurate than is required for clinical interpretation or treatment.

#### SUMMARY AND CONCLUSIONS

A "micro" modification of the Folin-Wu blood sugar which employs no special apparatus is described.

For use with this modification a procedure for preparing permanent colored standards covering a wide range of values is described.

The feasibility of the use of such standards is discussed and their accuracy compared with the macromethod and with clinical requirements.

The advantages of this micromethod used in conjunction with permanent standards in clinical practice is indicated.

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## A PHOTOELECTRIC DROP RECORDER\*

CLARENCE F. GRAHAM, M.D., AND F. STANLEY RANDLES, M.D., ALBANY, N. Y.

A COMPLETELY satisfactory drop recorder has not yet been invented, if one may judge from the frequent reports of new devices which appear in the scientific journals. So far three main types of apparatus have been described: (1) the direct contact form, in which the drop makes an electrical contact directly between metallic points; (2) the displacement type, in which the drop displaces a conducting electrolyte which establishes an electrical contact; (3) the mechanical contact type, in which the impact of the drop closes a circuit by some mechanical movement.

Apparatus of the first type is uncertain in its operation because of corrosion and gas formation on the contact points, and of course will not operate at all unless the drop is a fairly good conductor. The second type requires rather delicate apparatus and adjustment, and again gas formation at the electrodes will interfere with good conductivity. The third form will operate quite satisfactorily under certain conditions, but the inertia of the moving contact lowers the rate of response, and the inherent vibration frequency of the moving part may cause chattering and false recording.

The apparatus we wish to describe operates on a principle which has not yet been applied to drop counters, so far as we can learn, although it has been in satisfactory use for many years in similar applications. The falling drop is used simply to interrupt a beam of light actuating a photoelectric cell and the necessary amplifying system. The drop acts as a foreign body in the light beam, is not required to be either opaque or conducting, and has no contact with any part of the apparatus.

Fig. 1 shows the optical system used to obtain a concentrated beam of light that can be interrupted to a great extent by the falling drop. The lamp is an ordinary 3-candlepower tail-light bulb, conveniently fed by from a 6 to 8 volt bell-ringing transformer supplied with 115 volt alternating current. The condensing lens can be made of three ten-cent reading lenses put together, with a combined focal length of about 6 cm. If very small drops are to be recorded, the light beam can be diaphragmed to the sharpest possible focus by a stop, and with a little care the beam can be focussed so sharply that the movement of a single hair will operate the photoelectric system. For the convenience of a rigid mounting, the lamp, lens and photoelectric cell are set up on the same base. The cell is covered with a black paper jacket with a circular hole to admit the light beam to the target, and should be so placed

\*Received for publication, December 26, 1933.

This apparatus was demonstrated in operation at the Cincinnati meeting of the American Physiological Society in April, 1933.

that the circle of light beyond the focal point just covers the target of the cell from side to side. As little extraneous light as possible should reach the cell through the opening in its cover so that the drop may cut nearly all the light that reaches the cell.

The pipette is arranged so that the drop forms only 3 or 4 mm. above the focus of the light beam, and falls very little before it interrupts the beam. If the drop is formed too far above the beam, it may be deflected by air currents and cause unsteady operation. The most favorable location for the drop has been found to be just beyond the focal point, but its position can be adjusted by a few trials.

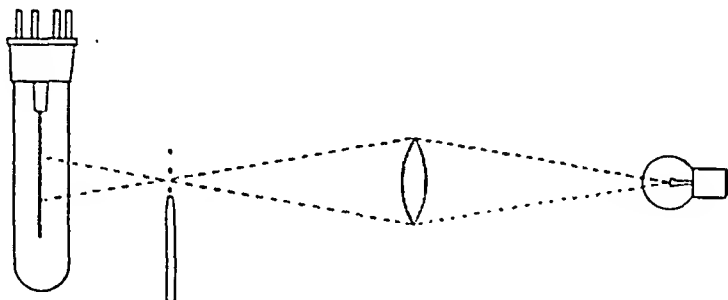


Fig. 1.—The optical system of the drop recorder.

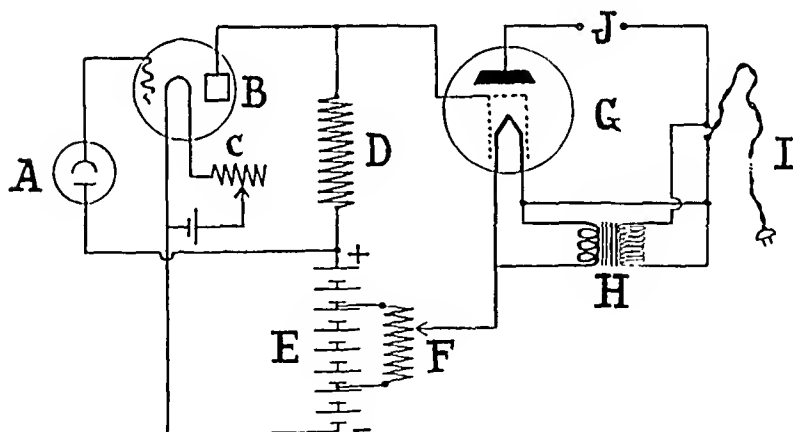


Fig. 2.—The electrical system of the apparatus.

Fig. 2 shows the electrical system employed to amplify the tiny current of the photocell. *A* is the photoelectric cell, which may be either an electron emission cell, or preferably a selenium tube such as the FJ-31 of the General Electric Company. *B* is an amplifying tube of the UX-240 type, supplied with filament current from a 6-volt storage battery through the 20-ohm rheostat *C*. The rheostat may be omitted with only a slight loss in the life of the tube. *D* is the load resistor of the amplifying tube, preferably of 200,000 ohms. *E* is the plate battery of 90 volts, made up of two 45-volt radio B batteries. As the current drain is slight, these batteries will run down very slowly. *G* is the heavy power tube, a G. E. thyratron FG-17. This peculiar

device is a mercury ionization tube which has the advantage of operating on raw 115-volt alternating current, with an output at this voltage of half an ampere. The filament-heating current for the thyatron is supplied at 2.5 volts and 5 amperes from a small step-down transformer *H*, which can be obtained at little cost from a radio supply store. No rheostat is needed in the filament circuit. The output of the thyatron is shown at *J*. The signal marker should be of high resistance with as many turns of wire as possible, since the internal resistance of the thyatron is much higher than that of a battery. *I* represents the electric cord and attachment plug for the supply of operating current from any 115-volt alternating current outlet.

The thyatron fits an ordinary four-pronged radio tube socket, and the connections to the grid and filament are made in the usual way. The plate connection, however, is made to the knob at the top of the tube in order to space the contacts wide for high voltage operation. The output of the tube is half-rectified alternating current.

In operation, the apparatus is plugged in, the light and amplifying tube turned on, and the thyatron is allowed to heat for about four minutes until the contained mercury is volatilized. With the light on the selenium tube, the potentiometer leads are connected to the B battery so that the thyatron can be turned on and off by rotating the potentiometer knob. With the thyatron just turned off, the pipette is placed in position so that the falling drop cuts the beam of light and causes the thyatron to flash on as each drop falls. When well adjusted, the thyatron should turn on for a 10 per cent reduction in light on the selenium tube, or a 30 per cent reduction on a caesium photoelectric cell. The sensitivity can be tested by moving a card into the light circle between the lens and the focal point, and estimating the amount that must be cut off before the thyatron flashes on. The amount of light cut-off required can be varied by turning the knob of the potentiometer. One lead from the potentiometer to the battery should be disconnected when the apparatus is not in use.

The amplifying system of the apparatus can be connected to the photoelectric cell by any reasonable length of wire, so that the selenium cell can be placed in a convenient location for the registration of the drops. The output circuit can be of any length. It is also a simple matter to adapt the apparatus for use as a time signal. The light beam can be interrupted by a swinging pendulum, or a vane on the rotating spindle of an electric clock can be arranged to pass through the beam at regular intervals. Many electric clocks of the synchronous motor type have a shaft which rotates once a minute, and a wheel can be mounted on the shaft with spokes of varying lengths so that time intervals of 10, 15, 20, 30, and 60 seconds can be obtained according to the distance from the center at which the light beam strikes the spokes. If time signals are to be dispensed to a whole class, it is advisable to pass the output of the thyatron into a 250-ohm relay, and send out secondary battery current at about 10 volts to the individual tables. In this way the disastrous effects of a short circuit on the thyatron can be avoided.

A word of caution may not be amiss. The apparatus as described would be classed as a moderately sensitive photoelectric apparatus, and as such demands the care usually given an instrument of precision. The whole set can be assembled in a couple of hours by anyone slightly acquainted with the wiring of radio apparatus. All connections should be cleaned and soldered, and the storage and B batteries should be kept up to their rated voltage at all times. If the voltage of the supply mains varies widely, the light may fluctuate enough to cause false recording. In such a case the lamp must be fed from a storage battery. The thyatron should never be short circuited, since the heavy ionic bombardment caused by a short circuit quickly ruins the filament. The thyatron will operate only on alternating current. On long runs the potentiometer may require slight readjustment as the vacuum of the tube diminishes, but otherwise there is nothing to be adjusted. With these few precautions the apparatus operates with little or no attention.

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### A MORE CONVENIENT MICROPROJECTION APPARATUS\*

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WILLARD S. HASTINGS, M.D., PHILADELPHIA, PA.

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FOR group study of microscopic preparations, whether in classroom or conference, the projection microscope has certain very obvious advantages; it can be used extemporaneously, with the same preparations that have been used for individual study and without the delay incident to photography, objects are seen in their accustomed colors, and by moving the slides about or changing powers the relations of, or variations in different portions of sections or other preparations are clearly and quickly demonstrated. In most institutions, however, the instrument is used but rarely. Aside from difficulties rising from flicker or inadequacy of the light source, much of the disappointment in it has seemed to be due to the experience that its use involves much lost motion and is time consuming. In the usual apparatus, slides must be picked up and slipped in a vertical position beneath clips or into a mechanical stage. They are moved about in an unfamiliar manner, and altogether the demonstration lags far behind the natural rate of the accompanying discussion.

To overcome this objection, the apparatus to be described was devised and has been used with much satisfaction in pathologic conferences. Ideally, such an apparatus should: (1) Provide for rapid, easy, and natural manipulation and interchange of slides. (2) Have a considerable range of magnification, changed with a minimum of time and effort. (3) Give approximately "critical illumination," with as few motions on the part of the operator and as near an approach to a positive setting for the more used powers as can be arranged. Most projection microscopes have been adaptations in mounting of more or less specialized variants of the familiar microscope stand. Our thought

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\*From the Jeanes Hospital.

Received for publication, January 4, 1934.

has been, on the contrary, in order to arrange for the first of the above desiderata, to start with the table top and build the microscope into it, making the table serve as the microscope stage. The microscope tube is kept in a vertical position by the familiar expedient of reflection of the light source and of the projected image. With this arrangement, a considerable number of slides may be interchanged purely by a sliding motion. The slide being demonstrated is moved exactly as when the microscope is used individually and

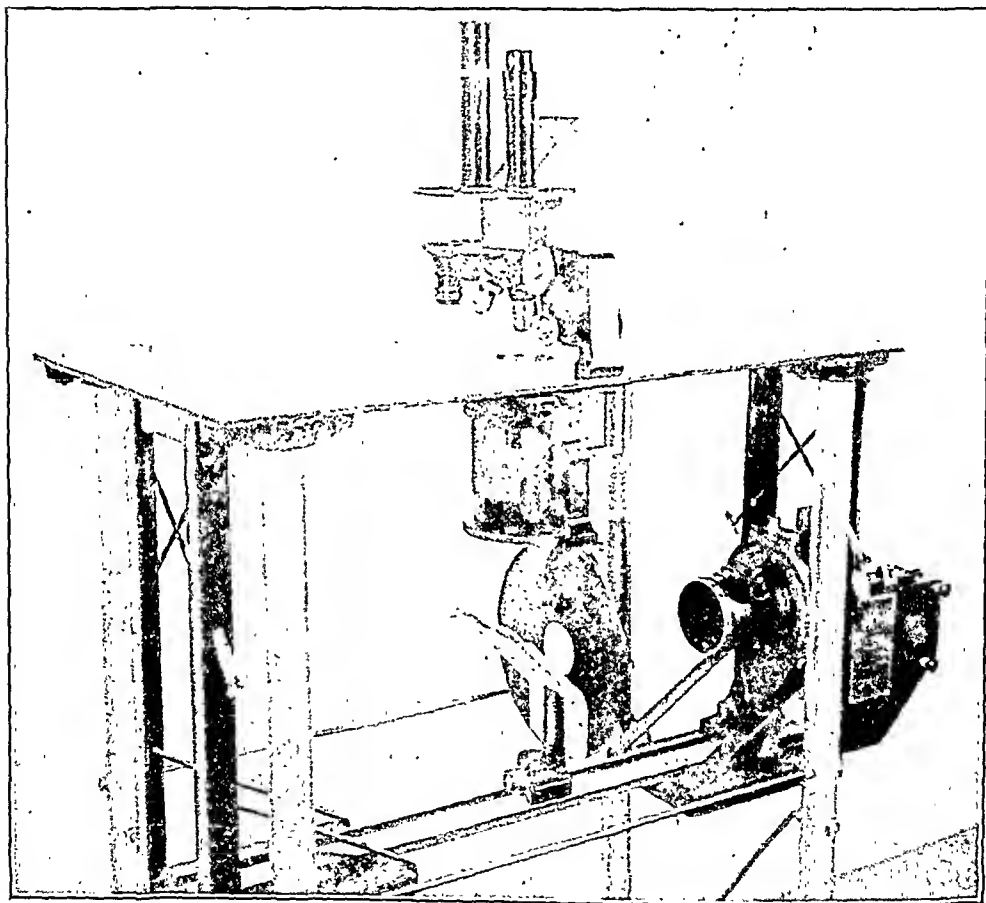


Fig. 1

gravity is an aid instead of a hindrance to the holding of a field once selected. In fact, clips are not desirable under most conditions, though holes have been provided for them and for a mechanical stage.

It has been constructed by taking apart a Bausch and Lomb "Large Projection Microscope" sufficiently to remove the stage. Holes were bored in a steel plate measuring  $25 \times 15$  inches, and tapped and provided with pins exactly to correspond with those in the original stage. The microscope was then reassembled, with the steel plate taking the place of the stage and serving as the support of the arms carrying the microscope above and the substage

below. The central opening was made  $1\frac{1}{4}$  inches in diameter, countersunk on top to provide for a blackened brass disk with a one-inch opening. Brass legs 42 inches long and suitably braced were attached to the four corners. Entirely out of contact with these legs is a support for a metal optical bed, suspended from the ends of the steel plate 15 inches below it. This is made rigid by diagonal bracing, and provision is made for horizontal adjustment at a right angle to its length by means of wing nuts on the braces across the ends. Since the end supports have been made to form an exact parallelogram, the surface of the optical bed remains parallel with the steel plate during any such change. An automatic feed arc lamp is attached to the optical bed at one end. With an 8 ampere rheostat and  $\frac{5}{16}$  inch cored carbons this is nearly free from flicker and wandering of the crater. Under suitable conditions, other light sources would probably prove satisfactory and be more convenient than the arc. A 45 degree mirror is supported on the optical bed below the axis of the microscope.

The substage, attached below the steel plate, carries a large condenser for use with microtessars or similar lenses, and above this two smaller condensers of differing numerical apertures on a swinging arm for use with microscope objectives. A recess must be drilled partially through the plate for the small condenser not in use, to permit the condenser in use to be elevated close to the slide for objectives of high aperture.

The stand has a  $5\frac{1}{2}$  inch revolving plate which carries a quadruple nose piece and openings for three low-power objectives and microtessars. Another similar plate has two tubes for eyepieces and a 45 degree first surface mirror, the latter for low-power projection without an eyepiece. A prism is used above the eyepieces to project the image in a horizontal plane; it can be turned in any direction. Since the stand was intended for use in another manner, the mirror is mounted to throw the beam of light "backward" across the supporting arm. The operator is thus obliged to stand in an inconvenient position. By filing away a portion of a corner of its support, and sacrificing one of the eyepiece tubes, the mirror can be reversed and the operator can then be in a natural position behind the stand. (This was not yet done when the accompanying photograph was made.)

With casters on the legs, the prism is about 54 inches above the floor. When further elevation of the projected image is desired, the entire apparatus is tipped backward slightly. With small groups this is not often necessary. Hinging of the top would provide for elevation with greater stability, but it is very desirable to avoid cross structural elements which would interfere with easy manipulation of the substage.

Thanks are due to Mr. J. I. Wexlin, local representative of Bausch and Lomb, for suggestions regarding light sources and suitable optics for projection.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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**MALARIA, Blood Groups and Therapeutic, Polayes, S. H., and Derby, I. M. J. A. M. A. 102: 1126, 1934.**

A series of 127 cases of dementia paralytica treated with malarial blood was studied in order to determine (a) the effect of the injection of compatible or incompatible blood on the incubation period, (b) the occurrence of immediate or delayed reactions to incompatible malarial blood, and (c) the frequency of occurrence of so-called primary fever. The following conclusions may be drawn:

1. The average incubation period is 4.30 days when the malarial blood injected is compatible and 8.18 days when it is incompatible with the recipient's serum. That this difference is significant was proved by statistical analysis.

Wide variations of the incubation periods were noted in the group of patients receiving compatible as well as in those receiving incompatible blood. These differences may be ascribed to factors other than compatibility or incompatibility of blood.

2. With regard to untoward postinoculation reactions, it was not possible to determine with any degree of certainty that they were due to incompatibility between the donor's and the recipient's blood. Other possible factors may be responsible for these reactions.

3. Primary fever occurred in only 15 per cent of the patients receiving compatible malarial blood. These figures are much smaller than those found by previous investigators. The difference is perhaps due to the fact that it is difficult to recognize this phenomenon because the postinoculation temperature curve is frequently too irregular to conform rigidly to the definition of primary fever.

**B. C. G., Spontaneous Occurrence of a Non-Acid-Fast Form in a Culture of, Sidenberg, S. S., and Ecker, E. E. Am. Rev. Tuberc. 29: 571, 1934.**

A nonacid-fast variant of B. C. G. was discovered in a culture obtained from Paris on February 4, 1932.

The variant possessed the growth properties of the tubercle bacillus and reverted to the acid-fast form when grown on various tubercle bacillus media over a sufficient lapse of time.

The variant showed none of the characteristics of a contaminating organism.

Guinea pig inoculations yielded cold abscesses at the site of the injection. Acid-fast organisms were recovered from these abscesses.

Although the B. C. G. is not a fixed form of microorganism and though it may yield a variant as described, the variant was devoid of pathogenicity for guinea pigs.

**B. TYPHOSUS, Evidence That *B. alkalescens* (Andrewes) May Be a Variant of, Gilbert, R., and Coleman, M. B. Am. J. Pub. Health 24: 449, 1934.**

It has been shown that strains of bacteria having the properties of *B. alkalescens* are present in approximately 1 per cent of all specimens submitted for bacteriologic examination for evidence of enteric disease. Clinical and epidemiologic data suggest a close relationship to the typhoid bacillus.

In the study of specimens from patients recovering from typhoid fever and in the search for carriers, the presence of this type of microorganism seems of special import. Even though, in itself, it may have no pathogenic significance, the fact that in so many instances *B. typhosus* has been isolated from the same or other specimens from the individual concerned, makes a thorough search for this microorganism imperative, whenever strains of *B.*

*alkalescens* are found. When suspected typhoid carriers are concerned, it would seem very important to examine a large series of fecal specimens and, if possible, duodenal contents.

**PYURIA, Frequency of, in Anomalies of the Urinary Tract in Children, Begler, J. A.**  
Am. J. Dis. Child. 47: 780, 1934.

Anomalies of the urinary tract are of frequent occurrence, and the majority of them are congenital. In this series, so far as could be determined, none of the urinary obstructive lesions were due to acquired stricture caused by infection or inflammatory reaction in the ureteral wall.

Many such lesions go unrecognized for a number of years because of the lack of recognizable symptoms.

Obstructive lesions with urinary stasis may be present for years without infection and pyuria.

Since 50 per cent of the children with urinary stasis had pyuria, it appears that urinary infection in these children may be more frequent than in normal children, but that stasis, contrary to the usual belief, does not always lead to pyuria. When infection does take place it is exceedingly persistent, even becoming permanent, unless the obstruction is relieved.

Catheterization or the manipulations of urologic investigation invariably lead to infection when stasis is present.

A normal urine does not exclude the possibility of an obstructive lesion of the urinary tract.

**ENTERITIS, Relationship of Shigella Alkalescens to Other Members of the Shigella Group, Welch, H., and Mickle, F. L.** Am. J. Pub. Health 24: 219, 1934.

Cases of dysentery-like diseases and others apparently caused by *Shigella alkalescens* are reported.

The relationship of *Shigella alkalescens* to other members of the dysentery group is demonstrated by means of agglutination, agglutinin absorption and the Schwartzman phenomenon.

Shiga antifiltrate horse serum neutralizes in rabbits and mice the toxic effect of *Shigella alkalescens* filtrates.

This investigation indicates that *Shigella alkalescens* should be included among those organisms in the *Shigella* group capable of causing disease in man.

**FOOD POISONING, Suggested Laboratory Procedures for Use in Determining the Cause of, Koser, S. A.** Am. J. Pub. Health 24: 203, 1934.

#### *A. First Examination:*

1. Prepare a stained smear (Gram stain) directly from the foodstuff, preferably the liquid portion.

2. With a loopful of the foodstuff streak in succession 2 agar plates, preferably a meat infusion agar. With another loopful streak 2 more plates of either Endo or eosin-methylene blue agar. Incubate for from eighteen to twenty-four hours at 37° C.

NOTE: If the only sample submitted is an empty jar or can, the interior should be thoroughly washed out with a few c.c. of sterile salt solution or sterile broth. The washings can then be used for the examination.

3. Inoculate a tube of broth, preferably prepared from meat infusion, with a small amount of the sample. Incubate at 37° C.

If the poisoning is suspected or definitely known to be botulism the following steps should be included:

4. Inoculate each of 3 tubes of ground meat or beef heart medium with approximately a gram or 1 c.c. of the sample. Ground lean beef, beef heart or veal is tubed with the infusion from the meat so that the layer of ground meat occupies about half of the column of the liquid. The final pH should be from 7.2 to 7.6.



Immediately after inoculation 2 of the 3 tubes should be heated to 80° C. for twenty minutes to destroy vegetative cells. If an anerobic jar is not available the 3 tubes should be layered with about 1 c.c. of sterile vaseline to form a seal. Incubation should be at 35° to 37° C.

5. A portion of the sample should also be used to test for the presence of toxin. Inject subcutaneously at least 2 guinea pigs or 2 white mice with a portion of the food. In most cases it will be necessary to centrifuge the sample before injection to get rid of gross contamination. Use 0.5 to 1.0 c.c. of the supernatant fluid for injection. Where abundant toxin formation is suspected and sufficiently large sample is available, it is desirable to feed portions to 2 guinea pigs.

Should death of any of the animals occur, controls with antitoxin of types A and B should be included. Inject subcutaneously 2 more animals with a protective dose of Type A antitoxin (usually 1 c.c. is sufficient) and an additional 2 with Type B antitoxin. These 4 animals should then be injected with the foodstuff in the same way as the original 2 which did not receive antitoxin. If the antitoxic sera are not at hand, these controls should be performed as soon as they are available.

### *B. Subsequent Examinations:*

1. Microscopic examination of the stained smear will give an idea of the relative abundance of bacteria in the product and of the morphologic types present.

2. Examine the Endo or eosin-methylene blue plates for the presence of colonies resembling those of the paratyphoid, typhoid, or dysentery groups. If suspicious looking colonies are found, fish several to either Russell medium or to fermentation tubes of dextrose, lactose, and sucrose broths. If such colonies are not encountered, the plates should be incubated an additional twenty-four hours and reexamined. The identification of suspected intestinal pathogens requires additional procedure and is described in Section C.

Examine also the nutrient agar plates and make Gram stains of several representative colonies. Note especially if staphylococcus or streptococcus colonies are present in considerable numbers.

3. The purpose of using a tube of broth is to detect by enrichment and subsequent plating any *Salmonella*, staphylococci, or other types which might have been missed by direct plating. If the steps listed under A2 and B2 have yielded no information of value, streak Endo or eosin-methylene blue plates and nutrient agar plates from this broth culture. The examination of these plates should then proceed as given under item B2.

4 and 5. (If suspected botulism.) The condition of the animals which were injected under item A5 will afford preliminary evidence of the presence or absence of botulinum toxin in the food. This should not be supplemented by examination of the meat medium tubes inoculated under item A4.

These tubes should be incubated for at least three or four days. Note any macroscopic evidence of growth and prepare Gram stains from each tube. Note whether gram-positive bacilli, with or without subterminal spores, are present. Select one or more tubes for a toxicity test similar to that carried out with the original sample under item A5. The result of this will serve to confirm that secured with the food sample and at times it may be of

RUSSELL'S MEDIUM (24-HOUR READING)	FERMENTATION TUBES			
	DEXTROSE	LACTOSE	SUCROSE	
Acid and gas in butt, alkaline slant	Acid and gas	Negative*	Negative*	Similar to that produced by <i>Salmonella</i> group
Acid in butt	Acid	Negative	Negative (or occasionally acid)	Similar to Flexner dysentery
Acid in butt	Acid	Slow acid	Slow acid	Similar to Sonne dysentery

\*Incubation should be continued for at least a week or preferably two weeks to rule out delayed fermentation of these sugars. Delayed fermentation of lactose or sucrose is not typical of the *Salmonella* group.

great value in affording some additional information. In the event of a positive test the meat medium may be used for further purification and isolation of the culture, if this is desired.

*C. Checking of Suspected Salmonella or Other Intestinal Types:*

Fermentation results similar to those shown in the accompanying outline immediately raise the question whether the organism may be a member of the *Salmonella* group or one of the dysentery bacilli. Since there are many miscellaneous saprophytes which resemble the intestinal pathogens in these superficial aspects, a somewhat detailed procedure of checking is necessary. When a culture has been selected for further examination a Gram stain should be made to see whether one is dealing with a medium-size, gram-negative, nonsporulating rod.

**GRANULOCYTOPENIA, Primary, The Etiology of (Agranulocytic Angina), Madison, F. W., and Squier, T. L. J. A. M. A. 102: 755, 1934.**

The increase in incidence of primary granulocytopenia (agranulocytic angina) has paralleled the increase in the use of drugs containing amidopyrine and especially those containing amidopyrine with a barbiturate.

The disease has appeared most frequently in persons apt to be taking drugs: physicians, nurses, or those directly under the care of a physician.

In each of fourteen patients the onset of primary granulocytopenia was directly preceded by the use of amidopyrine alone or in combination with a barbiturate.

The mortality in a group of six patients who continued the use of drugs containing amidopyrine was 100 per cent. In a group of eight patients who did not continue the use of these drugs, only two died, and both of these died in the initial attack.

The administration of a single dose of amidopyrine to each of two patients who had recovered from the acute disease was followed by a rapid profound fall in granulocytes.

One rabbit given allylisopropylbarbituric acid with amidopyrine (allonal) by mouth in relatively large doses showed an abrupt drop in granulocytes and died on the thirtieth day. Preceding death there was complete absence of granulocytes in the peripheral blood. Seventeen other rabbits given allonal or amidopyrine showed no significant change in the blood picture.

The authors believe that amidopyrine alone or in combination with a barbiturate is capable of producing primary granulocytopenia in certain individuals who have developed sensitivity to the drug.

They also believe that the appearance of primary granulocytopenia following the use of such drugs may be the result of an allergic or anaphylactoid drug reaction.

**B. PERTUSSIS, Cough Plate Examinations for, Kendrick, P., and Eldering, G. Am. J. Pub. Health 24: 309, 1934.**

Cough plate diagnostic service for pertussis has been available continuously for about a year at the Western Michigan Division Laboratory of the Michigan Department of Health, Grand Rapids. It is proving a practicable procedure under the conditions existing there.

During the past four months of this service 23 per cent of the positive diagnoses have been made within forty-eight hours after the plates have reached the laboratory, 75 per cent within seventy-two hours, and 91 per cent within four days.

The laboratory findings are being used by the City Health Department in obtaining earlier diagnosis of pertussis and thereby, it is hoped, more effective isolation of cases in their most infective stage. The possible applicability of the results to the problem of release is under study.

In agreement with the reports of most other authors, *B. pertussis* could be isolated from relatively few patients after the fourth week of disease. A patient with whooping cough of more than four weeks' duration, who can be shown to harbor *B. pertussis*, is defined tentatively as a post-whooping cough or convalescent carrier.

One hundred thirty-six cultures isolated from whooping cough patients, at times ranging from before onset to the thirty-fifth day of the disease, were found to fall into the same serologic group.

**ULCER, Oral Administration of Metaphen in the Treatment of Gastric and Duodenal,**  
 Trippe, C. M. *Ann. Med.* 6: 901, 1933.

Metaphen, in 1:500 solution, was given orally to patients with symptoms of chronic abdominal distress in the dose of 4 c.c. three times a day with very gratifying results.

The material presented included an analysis of 82 cases, 26 of gastric and 56 of duodenal ulcers. Complete x-ray studies were made of 27 of these cases; diagnosis was also confirmed in many cases by test meal and microscopic study of gastric contents.

Relief from pain was obtained in practically all cases in an average of three days' time. No toxic effects were ever observed.

Complete disappearance of gastric and duodenal ulcers consequent upon treatment with metaphen has been demonstrated by means of x-ray studies, made before and after treatment.

A possible explanation of the action of metaphen, primarily a bactericidal agent, may lie in the part played by infection in the evolution of ulcers of the type considered, as has been recently emphasized by several authors.

**SPORE STAIN, Modification of Dorner's, Snyder, M. A. *Stain Techn.* 9: 71, 1934.**

(1) Make a thin film of the organisms on a slide. (2) Cover with a small piece of blotting paper and apply several drops of freshly filtered Ziehl's carbol fuchsin. (3) Allow preparation to steam five to ten minutes on a hot plate, keeping the blotting paper moist with carbol fuchsin. (4) Decolorize instantaneously with 95 per cent alcohol and wash with water. (This step may be omitted if the spores do not hold the color well, but in general a neater looking preparation is obtained with its use.) (5) Apply a drop of saturated aqueous nigrosin and spread evenly and thinly over the smear. Allow to dry quickly on the hot plate.

The results obtained (spores red and the vegetative parts of the cells almost colorless) are very similar to those of the unmodified Dorner technic. The procedure is simpler and quicker and is more successful in many cases. The above technic gave good results with spore-formers identified as the following: *Bacillus megatherium*, *B. niger*, *B. cereus*, *B. mycoides*, and with some cultures of *B. subtilis* (Marburg strain). The unmodified Dorner method is quite unsatisfactory for the last named organism; this modified technic is better, but does not give uniformly good results with all strains.

**FOCAL INFECTION, Necessity for Revising the Common Conception of, Solis-Cohen, M. J. A. M. A. 102: 1128, 1934.**

The common conception of focal infection concentrates attention on the infected tissue rather than on the infecting bacteria.

When the physician speaks of eradicating a focus of infection, what he really means is removing infected tissue, such as tonsils or teeth, or draining and cleaning out an infected cavity, such as a nasal accessory sinus.

Infecting bacteria seldom are confined to a circumscribed area of diseased tissue but are present also on adjacent tissues, which may be apparently free from disease.

Enucleating of diseased tonsils does not necessarily remove the focus of infection, because the infecting organisms may still remain in tonsillar stumps, in recurring tonsillar tissue, in infratonsillar lymphoid tissue, and on the apparently normal mucous membrane of the tonsillar fossae and the nasopharynx.

A focus of infection in a nasal accessory sinus is not necessarily eliminated by treatment or operation, because infecting bacteria may still persist there.

Such expressions as "removal" or "elimination" or "eradication" of the focus of infection should be avoided when one is describing tonsillectomy or sinus treatment or operation.

The conception of focal infection should stress the bacterial element and regard the causal germ as the chief infecting agent.

Surgery alone cannot possibly remove all the infecting organisms, which can be overcome only by the patient's defensive forces.

The elimination of a focus of infection requires the production of bactericidins and other antibodies to destroy the infecting bacterin and render their toxins harmless, which can be aided by hygienic measures but in many cases needs the artificial stimulation of a potent vaccine containing the proper antigens obtained from the infecting bacterin.

The pathogen-selective culture selects the etiologically important organism from a mixed culture, and the vaccine made from it contains both the endotoxins and the soluble exotoxins of the infecting bacterin.

When, in addition to the extirpation of accessible and removable diseased tissue that has become a favorable soil for the propagation of the infecting germs, the required specific antibodies have been produced in sufficient quantities to overcome the infecting organisms and their toxins, the focus of infection can truly be said to be eradicated at both the primary and the secondary foci.

**TUBERCULOSIS, An "Acid-Fast" Other Than Koch's Bacillus Cultivated From Sputum,**  
Cummins, S. L., and Williams, E. M. *Tubercle*, London 15: 49, 1933.

The patient was a woman suffering from acute pulmonary disease, which had come on shortly after a confinement. The picture was that of acute phthisis, but the sputum had been reported negative on several occasions. The authors found in a sample of sputum numerous acid-fast bacilli, scarcely distinguishable from tubercle bacilli but showing a few curious "balls" of curved rods, which were sufficiently peculiar to raise the question of an unusual morphologic type. From a culture a growth was obtained and from another sample of the sputum sent to Griffith six weeks later the same organism was cultivated. Whether the germ was merely an adventitious saprophyte or the causative organism of the severe pulmonary disease from which she suffered must remain open to question, as opportunities for further biologic tests were refused. The authors emphasize the fact that the finding of acid-fast bacilli in the sputum is not necessarily positive proof of tuberculosis. The importance of cultural verification in all doubtful cases cannot be stressed too much.

**HEMOPHILIA, Negative Effect of Prolonged Administration of Ovarian Substances in,**  
Stetson, R. P., Forkner, C. E., Chew, W. B., and Rich, M. L. *J. A. M. A.* 102: 1122, 1934.

The coagulation time of the blood of seven patients with hemophilia was observed repeatedly during treatment with ovarian substance or estrogenic substance. Large amounts of one or another of eight different preparations were given over periods of from twenty-eight to eighty-one days. In no instance was the coagulation time of the venous blood found to be depressed as the result of such therapy. Theelin (estrogenic substance) administered subcutaneously to two patients for from three to seven days neither stopped bleeding nor reduced the clotting time.

Estrogenic substance was demonstrated consistently in the urines of these patients both under ovarian therapy and during control periods. No correlation could be established between the quantity of estrogenic substance excreted in the urine and the fluctuations of the blood coagulation time.

No clinical improvement was noted which could be attributed to ovarian therapy. In four of the patients hemarthroses developed without obvious trauma or infection late in the course of treatment.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

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### The International Medical Annual\*

WHILE it is true that the progress of medicine is reflected in the current medical literature and that the articles of today are often, as it were, the textbooks of the future, it is also true that few are either in a position or have the time to follow the enormous mass of medical publications which fill the journals every year.

This was the genesis of the International Medical Annual, now in its fifty-second year, which has for its purpose the presentation of a conspectus of current medical literature.

The purpose of these volumes is too familiar, and the issues each year too well known to require extended comment. The present issue maintains the standards set by its predecessors.

The plan of the work, which is, as it were, a dictionary of practical medicine, when coupled, as it is, with a comprehensive index, enables rapid reference to the contents; what cannot be found readily in the body of the text is easily located in the index so that the practitioner in search of specific information may find it quickly accessible.

While the book is primarily a review and survey of the literature of the year, and hence limited as to content to what has or has not been published, many special reviews and articles are included independently of the literature.

The annual can be commended as a comprehensive survey presented in a readable and usable manner.

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### Pathogenic Microorganisms†

THIS book needs no introduction, having long been an outstanding standard text.

As heretofore the volume is divided into three main sections: Part I concerned with the general characteristics and methods for the study of pathogenic microorganisms; Part II discussing the pathogenic microorganisms individually; and Part III ("Applied Microbiology"), presenting methods for the examination of water, air, soil, shellfish, milk, and a practical discussion of disinfection and sterilization.

This tenth edition represents a thorough and comprehensive revision bringing the volume in accord with all the recent developments in this field.

Among the changes are a revision of the chapter on microscopic technique; a discussion of the newer types of pneumococci and the newer studies of the colon-typhoid group; a discussion of the present status of B. C. G. vaccination; recent studies in bacterial metabolism; recent studies in the preparation of diphtheria toxin and an evaluation of the flocculation test for the determination of its antigenic value; a revision of the section on spirochetes and of the section on filtrable viruses; newer studies in the examination of milk, water, and shellfish, and a revision of the chapter on hypersensitiveness.

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\*The International Medical Annual, A Year Book of Treatment and Practitioner's Index. Edited by H. Lethbey Tidy and A. Rendle Short. Cloth, 579 pages, 69 plates, 101 drawings. William Wood and Co., Baltimore.

†Pathogenic Microorganisms. By William Hallock Park, M.D., Professor of Bacteriology and Hygiene, University and Bellevue Hospital Medical College, and Anna Wessels Williams, M.D., Assistant Director, Bureau of Laboratories, Department of Health, New York City. Cloth. Ed. 10, pp. 867, 215 engravings, 11 color plates. Lea and Febiger, Philadelphia.

Included, also, are discussions of the practical experience of the authors with active immunization against diphtheria and scarlet fever and the results of recent studies in yellow fever, undulant fever, and poliomyelitis.

The familiar summary table has been extensively revised. This is such a useful form of rapid reference that one wishes it were in sections which could be removed, mounted, and made readily available in the laboratory.

This book, like its predecessors, may be recommended in the highest terms and without qualification as one of the most valuable texts in this field, not only for the laboratory worker but for all who are interested in medicine and allied subjects. Certainly no hospital, board of health, or clinical laboratory can afford to be without it.

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### An Introduction to Practical Bacteriology\*

THIS, the fourth edition of a well-known laboratory manual, has been completely revised to include recent developments in the field of bacteriology.

The volume retains its original purpose as primarily a guide to the practical laboratory study of bacteriology and consists of three main sections. Part I is introductory and in a brief and general way discusses the biology of bacteria and immunity in its relation to practical bacteriology; Part II is devoted entirely to bacteriologic and serologic technic; while Part III presents a description of pathogenic and commensal organisms, as well as the methods applicable to the bacteriologic diagnosis of various infections.

While intended primarily for the student the volume may well be considered a useful reference work for the physician and laboratory worker in general. As is commonly true of European texts, while the American literature has not been neglected, the references are predominantly European in origin. This does not, however, detract from the general usefulness of the work.

This book may be recommended to all who are interested not only in bacteriology per se but also in its practical application to the study of disease.

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### Treatment of the Commoner Diseases†

THIS book is based upon ten lectures constituting a postgraduate course of instruction and, as the title shows, is concerned with those disorders commonly encountered in general practice.

Although in this age of specialism the fact appears at times to be somewhat lost sight of, it is nevertheless true that no specialty demands the comprehensive knowledge and ability essential to the efficient conduct of a general practice. For the general practitioner, to be truly efficient, should be, first of all a specialist in diagnosis.

Few men have had a more comprehensive experience or are better fitted to write such a volume as this than Professor Barker, whose task, as outlined in the preface, has been well performed.

The volume is in no sense a formulary. Very wisely, Professor Barker, emphasizing its essential importance, had discussed the signs, symptoms, and diagnosis of the commoner diseases, embodying a comprehensive survey of the important and useful contributions which have appeared in the recent literature.

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\* *Introduction to Practical Bacteriology*. By I. J. Mackie, M.D., Professor of Bacteriology, J. E. Mc Cartney, M.D., Director, Research and Pathological Ed. 4, cloth, 504 pages. William Wood & Co., Baltimore.

† *Treatment of the Commoner Diseases*. By Lewellys F. Barker, Professor Emeritus of Medicine, Johns Hopkins University. Cloth, pp. 319. J. P. Lippincott Co., Philadelphia, Pa.

Throughout the volume there appears, of course, the therapeutic experience garnered from his own years of practice but the main purpose of the volume is to afford the practitioner an opportunity to become familiar with recent advances and to suggest sources for collateral reading in those subjects in which his particular interest may be centered.

The book may be recommended as comprising in small bulk a wealth of information clearly put and readily applicable to the problems of the practitioner at large.

### Brucella Infections in Animals and Man\*

THAT the disease once known as "Mediterranean fever," the cause of which was first described by Bruce in 1886, Bang's "abortion disease" in cattle, the cause of which was first described by Bang in 1897, and the very similar disease in sows first described by Traum in 1914, are all the result of infection with a single bacterial species, the Brucella, and that all three of these organisms are the cause of the disease in man known as undulant fever, is now a matter of common knowledge.

Undulant fever, moreover, as has been shown by the work of many investigators, is now recognized as a common and widespread infection which, because of its chronicity and manifestations, is of great importance.

The present manual, concerned principally with the methods of laboratory diagnosis applicable to its recognition, is a most timely publication which should be gratefully received by both laboratory worker and physician at large.

The author, Dr. Huddleson, has long been known for his extensive and varied studies in this field so that the text may be regarded as authoritative and based upon first-hand knowledge.

The idea of the work is to present the fundamental information concerning the disease and laboratory methods found satisfactory for its study, and to discuss these in such a fashion that the laboratory worker will be able to utilize them and the clinician will be able to interpret their results.

That this objective has been achieved the book itself is evidence.

The thoroughness with which the subject has been covered is shown by the appended chapter headings: The Genus Brucella; Methods of Isolating Brucella; The Pathology of Brucella Infections; Serological Methods of Determining Brucella Infections; Allergic Methods of Determining Brucella Infections; Method of Determining Brucella Infections by Measuring the Opsono-Cytoplagic Power of the Blood; and Methods of Differentiating the Species of the Genus Brucella.

A bibliographical as well as general index add to the value of the book which will, without doubt, become the standard reference in connection with this disease.

### Recent Advances in Medicine†

THIS, the seventh edition of this well-known book, has undergone extensive revision with the resultant addition of over one hundred pages of new material and seven new figures.

As before, the purpose of the book is to correlate the more recent advances in medicine as concerns the clinical and laboratory investigation of disease and, also, to a somewhat lesser extent, in the field of therapeutics.

\*Brucella Infections in Animals and Man: Methods of Laboratory Diagnosis. By I. Forrest Huddleson, Dept. of Bacteriology and Hygiene, Michigan State College. Cloth, pp. 108, 24 figures. The Commonwealth Press, New York.

†Recent Advances in Medicine, Clinical, Laboratory, and Therapeutic. By G. E. Beaumont, M.D., Physician, Out-Patient Department Middlesex Hospital, etc., and E. C. Dodds, M. V. O., Professor of Biochemistry, University of London. Ed. 7, cloth, pp. 485, 59 illustrations. P. Blakiston's Son & Co., Philadelphia, Pa.

The general manner of presentation remains the same; first, the description in some detail of the methods of investigation applicable to the formation of a diagnosis; second, a discussion of their significance and interpretation; and finally, some discussion of treatment.

Wherever a particular procedure is referred to, its technique is described in sufficient detail to enable the reader to attempt it although, as is unavoidable, for many of the procedures the clinician in general will have neither the equipment, the skill, nor the skilled help necessary.

Nevertheless, the value of any method of study, laboratory or otherwise, is enhanced by an understanding of the principles upon which it is based and of the various factors which may influence or interfere with its results, and, where the physician maintains his own laboratory, the book will be found a safe technical guide.

The needs of the clinician are kept in mind throughout the volume as witnessed, for example, by the careful discussion and tabulation of the high and low calcium diets, ketogenic diets, and the various dietetic schedules applicable to the treatment of diabetes.

It is questionable, however, whether the space given to the sanocrysin treatment of tuberculosis will be justified by the final status of this drug.

The volume well serves the purpose in view of furnishing to the student and clinician a useful reference work embodying the more recent advances in the field of general medicine.

This book may be recommended to the physician at large.

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### The Chemistry of the Hormones\*

THE underlying purpose and intent of this book is to furnish for the student a connected account of the chemical characteristics of the hormones, so far as these are at present understood and to present to the laboratory worker desirous of preparing active hormone fractions or who wishes to isolate a chemically pure hormone, detailed descriptions of the methods available.

The book is thus eminently practical both in concept and in the execution of the plan in view and within its nine Chapters can be said to present in a clear and satisfactory manner the present concepts of the chemistry of hormones.

The subjects covered are: the thyroid hormone; the parathyroid hormone; insulin; the pituitary hormones; the adrenal hormones; the male hormone, the female hormones; secretin; and plant hormones.

While, as indicated by the title, the volume is primarily concerned with the chemistry of hormones their physiological and clinical properties are also discussed. After each chapter is a list of the important references to the subject. Both a general index and an author's index are appended.

While the book is intended particularly for the student and laboratory worker by whom it should be heartily welcomed, it may well be utilized by the physician who desires to gain an understanding of these new therapeutic and diagnostic agents which seem destined to occupy a prominent place in the medicine of the future.

This book can be recommended as a useful, authoritative, and comprehensive survey.

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\*The Chemistry of the Hormones. By Benjamin Harrow, Ph.D., Associate Professor of Chemistry, College of the City of New York, and Carl P. Sherwin, M.D. Dr. P. H. Cloth, pp. 227. The Williams and Wilkins Co., Baltimore, Md.



# The Journal of Laboratory and Clinical Medicine

VOL. 20

ST. LOUIS, MO., OCTOBER, 1934

No. 1

Editor: WARREN T. VAUGHAN, M.D.

Richmond, Va.

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## EDITORIALS

### Skilled Labor?

WITHOUT doubt the first ostrich to thrust his head in the sand in order to hide from his enemies must have been greatly surprised on withdrawing it, to find them all about him licking their chops in ghoulis anticipation.

The medical profession today finds itself in a somewhat similar predicament, for its head, so to speak, has long been buried in the beautiful tributes paid to it in the literature of the past, such as the familiar homage of Robert Louis Stevenson and the less familiar but impressive saying of the philosopher Descartes who wrote: "If ever the human race is raised to its highest practicable level, intellectually, morally and physically, the science of medicine will perform that service."

Having basked in the pleasant glow of such approbation for lo! these many years, it is somewhat disconcerting to the physician now to find himself

accused of entire responsibility for the fact that disease, its aftermath, and its management connote expense; and to observe, also, that the management of disease, heretofore thought properly to be his business, is now the target for all manner of "experts" to shoot at, let the arrows fall where they may even to the making of a Roman holiday, as it were.

As long as there are human beings there will be injury and disease to incapacitate them, and as long as this is so some arrangement must be made to take care of them. These facts are incontestable but, somewhat strangely, what these measures shall be, how they shall be inaugurated, and in what manner they shall be carried out, are matters which are passing, if they have not already done so, from the hands of the medical profession.

On second thought this is not so strange, after all, for the medical profession has had no small share in the development of this situation. The problem itself, which recently has come to be spoken of as "medical economics," is as old as the profession but any discussion of it in the past has been somewhat frowned upon as somewhat undignified and tainted by commercialism; and despite its importance, immediate and personal, many have become somewhat weary of the apparently endless and, it must be said, sometimes pointless discussions of it without which few county society meetings can nowadays be held.

No one denies that the subject is one well worthy of discussion and concerted action but that concerted action toward a clear-cut and definite end will be initiated by the medical profession itself the experience of the past leads one to doubt, first, because medical economics has never before been the subject of frank discussion by physicians, and second, because, very probably, few doctors realize that the profession itself is in no small measure responsible for the situation in general. Perhaps a still further reason may be in the fact that the medical profession has long been obsessed with its ideals whereas the solution, if there be one, of the present problem will depend, not upon ideals, but upon what are usually spoken of as business principles, and the doctor has long prided himself upon being no business man.

The doctor, with some justice, may resent the bland usurpation of the right to regulate his profession by the various philanthropic, economic, efficiency, and other experts who appear to have taken over the job but there is little reason, after all, for excessive surprise at their appearance. For medicine has been preparing the way for them for centuries and has been building within itself a Frankenstein, although until present times there has been reluctance to recognize this and a tendency to smother the occasional voice which now and then has been heard crying in the wilderness.

Few, indeed, of the thousands of young men graduated in medicine have ever heard a word indicating that, while medicine is a profession of vital importance and inherent dignity, it is also a business, the business of controlling disease and, incidentally, of making a living for those who practice it.

There is nothing impossible in the association of an ideal with a business just as there is no disgrace in professing openly that one is earnestly engaged

in making an honest living but this latter aspect of medicine has somehow been kept under cover as being in some fashion incompatible with professional ideals.

And so, although responsibility for the care of the sick and indigent is not an individual but a community responsibility, the community has managed to saddle the doctor with the heaviest share of the load, as shown by the growth and expansion of hospitals and their activities until they are now actively engaged in the competitive practice of medicine.

Only recently has the doctor begun to realize this and he has yet to fully appreciate in some measure that it has come to pass and been made possible solely because of his connivance and active assistance without which, indeed, it could not be possible.

Poverty and disease are inescapable ills and certainly the responsibility for their existence is not primarily the physician's. Nevertheless when the poor are sick the doctor has to answer for them as if he were responsible for them.

Exposure and lack of food leading to lessened resistance are admitted factors in the incidence of disease. Even so, no one expects the builder to supply houses, the grocer to furnish food, or the clothier to hand out overcoats.

When disease occurs the community through taxes and contributions has furnished hospitals to which the poor can go.

The doctor, too, has through taxes and contributions also contributed to their upkeep, but it has not always been clearly appreciated that without his additional time-consuming, laborious, and personal services hospitals would be just buildings of no use per se to the sick.

Oddly enough, the public in general, even the intelligent public, is largely ignorant of the fact that physicians are not remunerated for the time spent in hospital free wards and dispensaries. Nor do even the doctors appreciate that, according to the statistics of one large city, 81 per cent of doctors are on hospital staffs and 67 per cent devote an average of seven or eight hours per week to dispensary work entirely without remuneration. Moreover, at least 35 per cent of patients receiving free care are not really entitled to it.

Although the business of treating disease is the function of the doctor, as it is conducted in the hospital, he has little or no control over how it shall be done and little if any voice in its management. The lay Board of Managers decides upon the rate to be charged in "free wards," money allocated to the hospital entirely, and decides who may or may not be charged for the professional services rendered. The lay social service worker decides whether or not a dispensary patient shall receive treatment.

Although it is the inalienable right of every physician to decide who shall be treated free, this is decided for him by clerks, not always efficient, of the social service departments subservient to lay boards of trustees and the superintendent, seldom a physician.

Hospitals are largely lay managed and lay controlled and the doctor is fast becoming merely the skilled labor employed by them, with this differ-

ence: that unlike all other forms of skilled labor he works for nothing and, apparently, likes it.

So hospitals have grown to mammoth industries based upon the uncompensated, unrecognized, and unappreciated work of the doctor who now finds them actively engaged in competition with him through the insurance schemes now advocated and partly in practice and the indiscriminate abuse of their free facilities.

It is small wonder that, in view of the fact that when the cost of medical care is analyzed the cost of *hospital care* bulks large indeed, that the doctor—a few of him, at least—is beginning to wonder if the worm might not venture a turn or two. At least, so they think in Australia where very recently a movement was inaugurated to secure concrete monetary recompense for the hospital work heretofore done for nothing by the doctor to his own gradually increasing, and now acute, disadvantage.

It would do hospitals and their managements no harm to consider just how they would carry on without the skilled assistance rendered freely by the doctor. The picture would be at least interesting.

—R. A. K.

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### The Bacteriologic Study of Food Handlers

ONE of the modern prophylactic provisions designed to lessen the incidence of diseases in the spread of which the carrier is regarded as of importance, is that which calls for the bacteriologic study of food handlers, a regulation which may be said, without injustice, to be more honored in the breach than in the observance.

Certain it is, however, that whenever an outbreak of enteric disease occurs there is a common tendency to demand a bacteriologic survey of all food handlers or persons engaged in other occupations which might render them a menace.

In view of the time, the labor, and the expense involved, it is well worth while to consider the results achieved, to balance these two factors, and to ascertain if the results warrant the effort expended.

Memory recalls an instance somewhat to the point which occurred during the World War.

In one of the infantry regiments of a certain division withdrawn from the line for rest, refitting, and replacement, there occurred a few cases diagnosed as typhoid fever and which, of course, were of interest. The history of the outbreak revealed that, despite the general orders to the contrary, this regiment on its way back from the front had neglected consistent chlorination of drinking water and had used the raw, untreated and commonly polluted waters of the French countryside. This information, coupled with the knowledge that antityphoid vaccination had not always been completed in the raw troops hastily sent overseas, seemed to furnish an explanation of the occurrence of a few cases of typhoid fever. But the powers that be

decreed that a search for carriers must be made and, accordingly, two officers, twelve enlisted men and a complete portable laboratory outfit traveled from the Central Medical Department Laboratories at Dijon to a small village "somewhere in France" and cultured an entire regiment at that time 1,800 strong.

The net result was the discovery of one carrier of *B. paratyphosus B.*, picked up while with the National Guard on the Mexican border.

The moral of this tale, if any, is that there may be more to the study and control of enteric outbreaks than isolated bacteriologic studies, to which fact attention has recently been called by Gilbert and Coleman<sup>1</sup> in discussing the examination of food handlers in state hospitals after outbreaks of typhoid fever had occurred in two.

In one hospital the examination of 116 specimens from 53 food handlers was without result. In the other, two carriers of *B. typhosus* and one of *B. paratyphosus B.* were found after the examination of 2,053 specimens from 813 food handlers.

The next result of the examination of 2,169 specimens, therefore, was the detection of three carriers one of whom, from a consideration of all the evidence, was probably not a carrier but recovering from an unrecognized infection.

As Gilbert and Coleman comment, assuming that, when large numbers are handled the average cost of an examination for *B. typhosus* is about \$2.00, the total cost involved in the detection of one carrier under the circumstances above, was \$1,500.

As they pertinently remark, since by the ordinary methods of examination of food handlers involving the study of one or two specimens every carrier will not be picked up, and since even when extensive studies are carried out only a relatively small percentage of positive findings will be encountered, a false sense of security results.

It should be emphasized, repeatedly, if necessary, that a study of environmental factors, careful history-taking and a thorough survey of all the factors accompanying and *preceding* the outbreak is an essential sine qua non in the study of enteric outbreaks. Not only is the information thus obtained of great value in itself, but, in addition to that fact it may be secured without extraordinary expenditure.

Moreover, it greatly enhances the value of the bacteriologic studies by enabling a selection of those to be examined, namely those who have had enteric disease, colitis, cholecystitis, etc., or whose immediate associates have had typhoid or paratyphoid infections.

Such a program, of course, is subject to modification as, for example, when cases of enteric disease occur along a milk route or among the patrons of a restaurant. In such a case, when the carrier is not found among those with a suggestive history, *where he should first be looked for*, then all the food handlers concerned should be examined.

When the outbreak under study suggests dysentery the problem is still further complicated by the fact that, as has recently become evident, organisms not included in the well-known dysentery group and heretofore regarded as nonpathogenic may be of etiologic importance.

Welch and Mickle,<sup>2</sup> for example, have recently reported the occurrence of dysentery-like diseases apparently caused by *Shigella alkalescens* and typhoid-like diseases accompanied by invasion of the blood stream by intestinal bacteria ordinarily without etiologic relationship to disease have been reported by others.

Bacteriologic surveys are too often ordered or deemed advisable without previous thorough studies of the outbreak which at times may definitely restrict the number of those to be examined while at the same time increasing the effectiveness of the laboratory study, and very often without a full realization of the expenditure of time, labor, and money involved.

West, Borman, and Mickle<sup>3</sup> in analyzing the examination of milk handlers over a period of seventy-seven months report that the total cost involved in the conduct of 91,257 examinations was \$48,048; the average cost of detecting each of the 71 carriers found being \$677.

While the per capita cost per consumer per annum was less than two cents in 1933, the cost per carrier varied from year to year, from a minimum of \$387 to a maximum of \$3,843.

It is profitable to consider, as suggested by Gilbert and Coleman, the more effective results to be expected if similar sums were expended in the pasteurization of products wherever possible, the improvement of sanitary facilities, the provision of training in personal hygiene, the removal from work and the treatment of food handlers who are obviously ill, the careful study of epidemiologic factors, and the examination of series of specimens from individuals *whose history or clinical manifestations warrant it*.

#### REFERENCES

1. Gilbert, R., and Coleman, M. B.: Practical Limitations in the Attempt to Control Enteric Disease by the Examination of Specimens Collected Without Regard to Clinical History or Epidemiological Evidence, *Am. J. Pub. Health* 24: 1, 1934.
2. Welch, H., and Mickle, F. L.: Relationship of *Shigella Alkalescens* to Other Members of the *Shigella* Group, *Am. J. Pub. Health* 24: 219, 1934.
3. West, D. E., Borman, E. K., and Mickle, F. L.: The Detection of Carriers Among Food Handlers in Connecticut, *Am. J. Pub. Health* 24: 493, 1934.

—R. A. K.

## CORRESPONDENCE

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Dear Sir:

I have read the article, "The Bactericidal Power of Blood," by Dr. Reuben Ottenberg and find that his results are not only at variance with those observations published by me in the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, October, 1933, but they do not agree with the authors cited in that paper. His conclusions appear, also, to be not in accord with that part of Cecil's technic which deals with the clotting of the blood in the isolation of streptococci from the blood stream.

The technic used by Dr. Ottenberg is open to criticism as mentioned by Topley and Wilson, "The Principles of Bacteriology and Immunity," Vol. I, page 170, and it may be that the results obtained were due to this technic.

Topley and Wilson (same page, etc.) state, "Other organisms, for instance most staphylococci and streptococci, are not killed by normal or by immune sera." Dr. Ottenberg's findings do not agree with this.

Recently, I found *Br. abortus* alive after three months in a defibrinated blood culture. At the time of obtaining this culture, the serum had an agglutination titer of  $\frac{1}{2000}$ . The culture had been kept at 35° C.

FREDERICK W. SHAW.

MEDICAL COLLEGE OF VIRGINIA,  
RICHMOND, VA.,  
MARCH 5, 1934.

# The Journal of Laboratory and Clinical Medicine

VOL. 20

NOVEMBER, 1934

No. 2

## CLINICAL AND EXPERIMENTAL

### AN ABRIDGED KEY TO THE GENERA OF PATHOGENIC FUNGI\*

FREDERICK W. SHAW, M.D., RICHMOND, VA.

IN PRESENTING this key for the use of clinical pathologists, the aim has been utility rather than an attempt at a natural system. No attempt has been made to place the ringworm fungi (Dermatophyta) in any systematic position, because this appears to be impossible at this time. Likewise, a few of the other pathogenic fungi have been classed as *Genera Dubia* for the same reason.

Under the *Genera Certa* have been placed those fungi whose characters are such that they may be classified, that is as far as this term may be used in relation to the *Fungi Imperfecti*.

This key contains the genera of most of the pathogens and the majority of the nonpathogens encountered by the medical bacteriologist.

#### A. DERMATOPHYTA. Parasitic and saprophytic forms in the skin and hair of man and other animals.

##### 1. Parasitic

##### a. Conidia of one kind in culture, fuseaux (Fig. 1-a)

##### 1. Epidermophyton

##### b. Conidia of two kinds

##### (1) Arthrospores present

##### (a) Aleuriopores present (Fig. 2-a); fuseaux absent

\*From the Department of Bacteriology and Parasitology Medical College of Virginia.  
Received for publication, January 20, 1933.



**2. Trichophyton**

- (b) Aleuriospores and hyphae with dichotomous subglobose or clavate apical branches

**3. Achorion**

- (2) Arthrospores absent or imperfect  
(a) Arthrospores absent; aleuriospores and fuseaux present

**4. Microsporon**

- (b) Arthrospores imperfect; fuseaux absent

**5. Endodermophyton****2. Saprophytic**

- a. Clusters of spores and mycelium in the skin

**6. Malassezia**

- b. Nodules on the hair

**7. Trichosporum****B. GENERA DUBIA**

1. Cause the white grain mycetomas

**8. Indiella**

2. Cause the black grain mycetomas

**9. Madurella**

3. Cause blastomycosis and coccidioidal granuloma

**10. Blastomycoides****C. GENERA CERTA (sensu lato)**

1. Mycelium nonseptate

*I. Phycomycetes*

2. Mycelium septate

3

3. Reproduce by thallospores

4

- a. Reproduce by hemispores

**11. Hemispora**

- b. Reproduce by conidiospores

5

4. a. Reproduce by arthrospores

6

- b. Reproduce by blastospores

7

5. a. Conidiophores present

1. Sterigmata (phialides) absent

*II. Conidiophorales*

2. Sterigmata present

*VI. Phialidineae*

- b. Conidiophores absent

1. Conidia are aleuriospores

*VII. Aleuriosporineae*

2. Conidia isolated and inserted directly on the hyphae

*VIII. Sporotrichiaceae*

6. Cultures form a scum on liquid medium and yeastlike growth on solid medium

*IX. Mycodermeae*

7. a. Dark colored 8  
     b. Bright or colorless 9  
 8. Conidia globose, ovate, oblong or short cylindric  
     a. Conidia one-celled, continuous  
         1. Conidiophores unbranched or sparsely so

**12. Dematium**

2. Conidiophores branched, treelike

**13. Haplographium**

- h. Conidia two-celled

**14. Cladosporium**

9. a. Mycelium present  
     1. Ascospores (Fig. 41) absent

**15. Monilia**

2. Ascospores present

**16. Endomyces**

- b. Mycelium not present

*X. Cryptococcaceae**I. Phycomycetes***A. Stolons present**

- a. Sporangiophores branched; columella ovoid, narrowed at the base

**17. Rhizomucor**

- b. Sporangioophores not branched

1. Sporangioophores arise at the nodes of the stolons

**18. Rhizopus**

2. Sporangioophores arise at the internodes

**19. Absidia**

- B. Stolons not present; rhizoids not present; hyphae branched**

**20. Mucor***II. Conidiophorales*

- A. Hyphae compact forming a globose, discoid or wart-shaped body.**

*III. Tuberculariaceae*

- B. Not as above**

1. Hyphae in loose cottony masses; conidia or hyphae dark, sometimes both

*IV. Dematiaceae*

## 2. Hyphae and conidia hyaline or bright colored

V. *Moniliaceae*III. *Tuberculariaceae*

Hyphae and conidia hyaline or bright colored; conidiophores mostly dendroid or verticillate; conidia more than two-celled, fusoid, curved, rarely in chains; sporodochia with bristles.

21. *Fusarium*IV. *Dematiaceae*

## A. Conidia in chains

22. *Alternaria*

## B. Conidia not in chains

## a. Conidia dark, exogenous

## 1. Conidiophores simple

23. *Acrotheca*

## 2. Conidiophores whorled much below apex

24. *Phialophora*

## b. Conidia mostly single and borne at tip, rarely clustered

25. *Acremoniella*V. *Moniliaceae*

## A. Conidia one-celled

## a. Conidia in heads

## 1. Conidiophores unbranched

26. *Cephalosporium*

## 2. Conidiophores branched

27. *Trichoderma*

## b. Conidia borne irregularly on hyphae

28. *Acremonium*

## B. Conidia two-celled or more

## a. Conidia two-celled

## 1. Conidiophores simple or nearly so; conidia capitate

29. *Cephalothecium*

## 2. Conidiophores irregularly branched

30. *Diplosporium*

## b. Conidia more than two-celled

31. *Allantospora*VI. *Phialidinae*

## A. Conidiophores inflated at apex

## a. Sterigmata unbranched

## 1. Sterigmata relatively long

**32. Citromyces**

2. Sterigmata relatively short

**33. Aspergillus**

b. Sterigmata branched

**34. Sterigmatocystis**

B. Conidiophores not inflated at apex; verticillately branched and unequal

**35. Penicillium***VII. Aleuriosporineae*

A. Hyphae pale, elongate

a. Sporogenous apparatus but little differentiated from the mycelium

**36. Acladium**

b. Sporogenous apparatus well differentiated

1. Aleuriospores colored

**37. Aleurisma**

2. Aleuriospores pale

**38. Corethrospis**

B. Hyphae dark; aleuriospores become dark

**39. Glenospora***VIII. Sporotrichineae*

For practical purposes there is but one genus in this group.

**40. Sporotrichum***IX. Mycodermeae*

A. Mycelium hyaline or bright colored

**41. Mycoderma**

B. Mycelium dark

**42. Torula***X. Cryptococcaceae*

A. Ascospores not formed

**43. Cryptococcus**

B. Ascospores formed

a. Spore membrane warty

**44. Debaryomyces**

b. Spore membrane smooth

1. Ascospores globose or ovoid

**45. Saccharomyces**

2. Ascospores lemon-shaped or hat-shaped

**46. Willia**

### 1. *Epidermophyton* Lang, 1879.

Hyphae and spores present in the lesions; pluriseptate spindles (fuseaux) present in the cultures; reproduction asexual, chiefly by the fuseaux. Fig. 1 organism and colony.

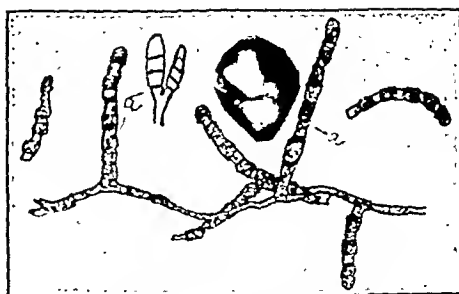


Fig. 1.—*Epidermophyton*.

### 2. *Trichophyton* Malmsten, 1848.

Hyphae and spores present in the lesions; mycelial and conidial spores in cultures; neither perithecia nor asci present. The conidia are aleuriospores (Fig. 2).

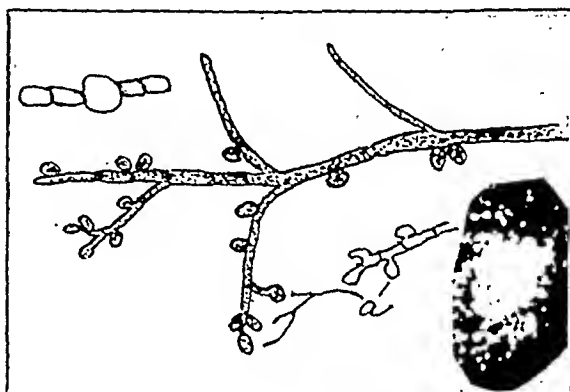


Fig. 2.—*Trichophyton*.

### 3. *Achorion* Remak, 1845.

Hyphae and spores in the lesions; spores situated apically and laterally on the hyphae, in cultures; arthrospores, aleuriospores and fuseaux in cultures in the form of clavate apical branches; without perithecia or asci (Fig. 3).

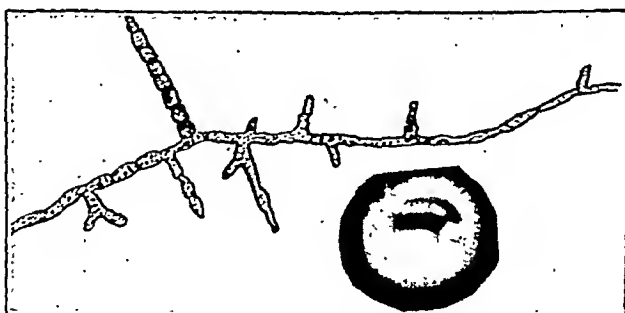


Fig. 3.—*Achorion*.

### 4. *Microsporon* Gruby, 1843.

Hyphae and spores present in the lesions; spores are small, and about from 2 to 3  $\mu$ . in diameter. Aleuriospores and septate or nonseptate fusiform bodies present in the cultures. Organism in a hair and colony in Fig. 4.

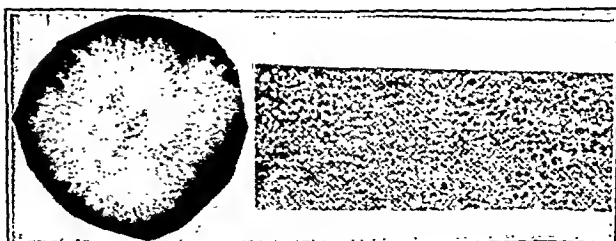


Fig. 4.—*Microsporon*.

5. *Endodermophyton* Castellani, 1909.

Aleuriospores rare and rudimentary; neither fuscaux nor accessory organs (spirally twisted mycelium) present; hyphae segmented; a few arthrospores may be formed.

6. *Malassezia* H. Baillon, 1889.

Members of this genus pathogenic for man have not been generally cultivated. In the tissues the organism appears as branched hyphae (segmented) and round or oval conidia, which may appear singly or in masses, and they may be smooth or have radial, longitudinal, or spiral striations (Fig. 5).



Fig. 5.—*Malassezia*.

7. *Trichosporum* Behrend, 1890.

Hyphae branched, hyaline then dark. Arthrospores produced. The cause of Piedra. Fig. 6 shows the lesion on the hair, the organism and colonies on dextrose agar.

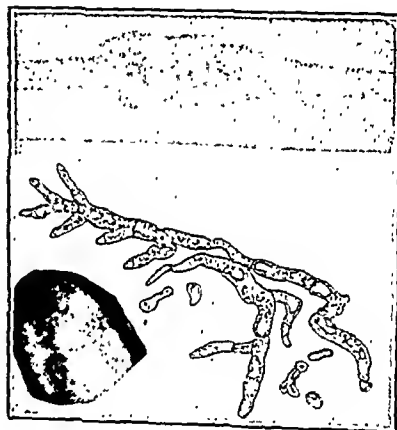


Fig. 6.—*Trichosporum*.

8. *Indiella* Brumpt, 1906.

Hyphae septate, branched, without black pigment. Sclerotia white or yellowish. Parasitic in the tissues of various animals. Not cultivated.

9. *Madurella* Brumpt, 1905.

Hyphae septate, reproduction by fragmentation of the hyphae. The spores are produced secondarily by binary division of the articles of which they are fragments. These fungi produce black maduromycosis. Fig. 7, organism and colony on dextrose agar.

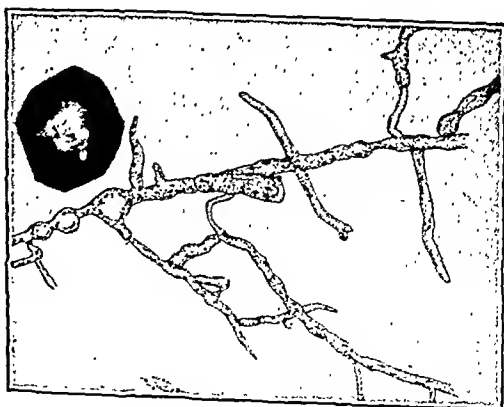


Fig. 7.—*Madurella*.

#### 10. *Blastomycoides Castellani*, 1927.

Budding forms appearing in the lesions as large roundish cells from 8 to 20 microns in diameter and even much larger, with the protoplasm containing a number of well-marked granules or spherules, and with the membrane showing a well-defined double contour; in dextrose agar cultures a large amount of mycelium is present. Fig. 8 shows the appearance of *Blast. immitis* in the tissues, and a colony on dextrose agar. Fig. 9 shows a colony, the growth magnified, and the *Blast. gilchristi* in the lesion.

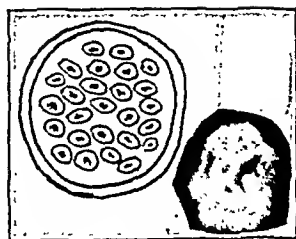


Fig. 8.—*Blast. immitis*.

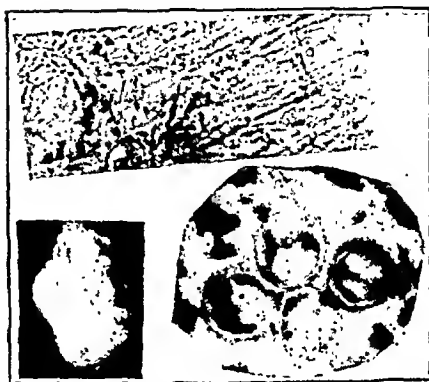


Fig. 9.—*Blast. gilchristi*.

#### 11. *Hemispora* Vuillemin, 1906.

Hyphae abundant, thin, septate, branched. Fertile hyphae branched at the base. These conidiophore branches are terminated by an ampulliform structure (protoconidium) which later is transformed into a series of sporelike segments (deuteroconidia) (Fig. 10).

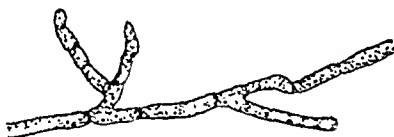


Fig. 10.—*Hemispora*.

#### 12. *Dematium* Persoon, 1797.

Sterile hyphae creeping, little developed or forming a turf; conidiophores erect, unbranched or sparsely branched, septate, with lateral conidial chains. Conidia spherical, or ovoid, at times held together by short connecting cells; one-celled, dark colored (Fig. 11).

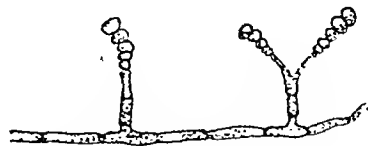


Fig. 11.—*Dematium*.

#### 13. *Haplographium* Berk. & Br., 1859.

Sterile hyphae creeping or obsolete; conidiophores erect, with treelike cluster of branches from which chains of spores arise. Conidia one-celled, spherical or ovoid, subhyaline, olive or dark colored (Fig. 12).

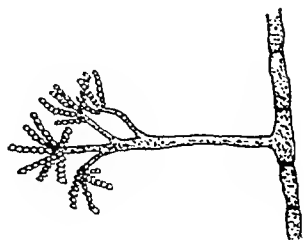


Fig. 12.—*Haplographium*.

**14. Cladosporium Link, 1816.**

Hyphae creeping, intricately branched; septate; olive colored. Conidiophores almost erect. Conidia in short chains or solitary, spherical and ovoid, at first one-celled, then usually two- or more-celled. Hormodendron is probably identical with cladosporium. Fig. 13 shows the hyphae and spores of cladosporium.

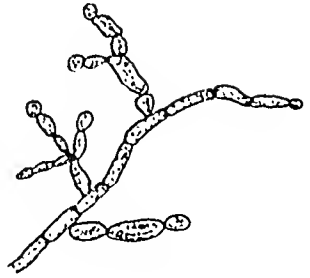


Fig. 13.—Cladosporium.

**15. Monilia Gemelin, 1791.**

The present tendency among medical mycologists is to include in this genus those forms which in their parasitic life form mycelial threads and budding forms, and in liquid cultures produce threads and budding forms, while on solid media the form is that of the yeasts, with very few mycelial threads. Asci are not formed. Generally produce gas from carbohydrates. Fig. 14 shows colony growth and the appearance of Monilia from a liquid culture.

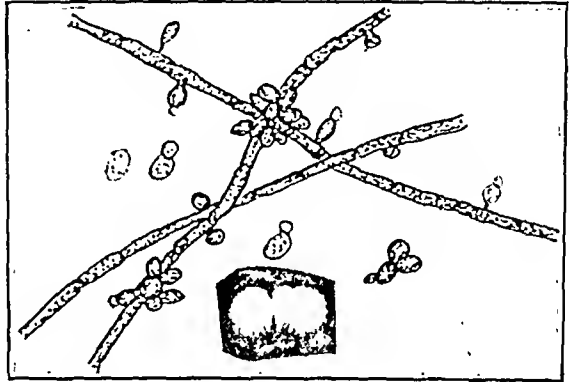


Fig. 14.—Monilia.

**16. Endomyces Reess, 1870.**

This genus has the same description as for Monilia, except that asci and ascospores are formed. Fig. 15 shows the organism from a liquid culture, a colony on maltose agar, and an ascus with four ascospores. The ascus is drawn about four times the relative size of the vegetative organism.

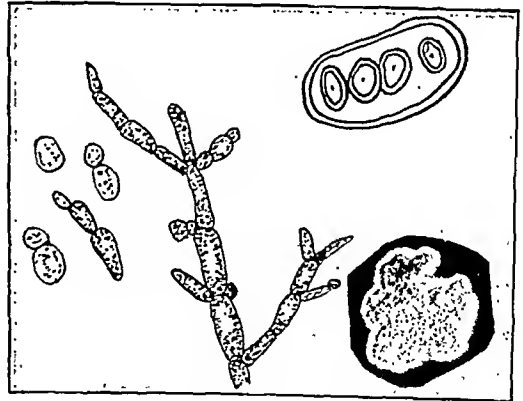


Fig. 15.—Endomyces.

**17. Rhizomucor Lucet and Costantin, 1900.**

Rhizoids and stolons present; sporophores branched; columella ovoid, narrowed at the base (Fig. 16).

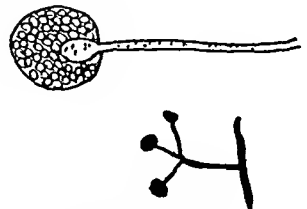


Fig. 16.—Rhizomucor.

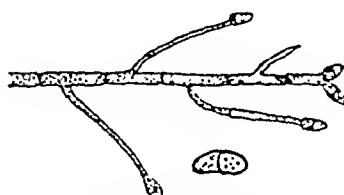


29. *Cephalothecium* Corda, 1842.

Hyphae prostrate; conidiophores erect, simple, septate; conidia apical, subcapitate, pyriform to oblong, hyaline. Identical with *Trichothecium* Link, 1824 (Fig. 27).

Fig. 27.—*Cephalothecium*.30. *Diplosporium* Bonorden, 1851.

Sterile hyphae creeping; conidiophores irregularly branched. Conidia ovoid or oblong, two-celled, hyaline (Fig. 28).

Fig. 28.—*Diplosporium*.31. *Allantospora* Wakker, 1895.

This genus is distinguished from *aeremonium* by the conidia in the form of sausages with rounded ends. These conidia are often grouped in a head. Conidia more than two-celled, imbedded in mucus, hyaline or bright colored. Conidiophores distinct from the conidia, segmented.

32. *Citromyces* Wehmer, 1893.

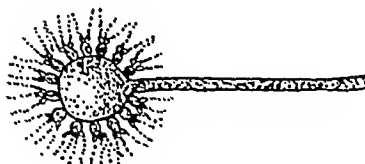
Hyphae branched, septate; conidiophores unbranched, distended at the apex in the form of a bladder or globule; conidia without mucus matrix (Fig. 29).

Fig. 29.—*Citromyces*.33. *Aspergillus* Micheli, 1729.

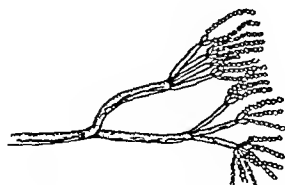
Sterile hyphae creeping, branched, septate; conidiophores erect, inflated at the apex, unbranched; conidia in chains, terminal on sterigmata. Sterigmata relatively short. Fig. 30, showing sterigma (s), and conidia (c).

Fig. 30.—*Aspergillus*.34. *Sterigmatocystis* Cramer, 1869.

Sterile hyphae branched. Conidiophores erect, unbranched, distended at the apex in the form of a bladder or globule; sterigmata branched, with occasional simple forms; chains of conidia formed at the apex of the sterigmata (Fig. 31).

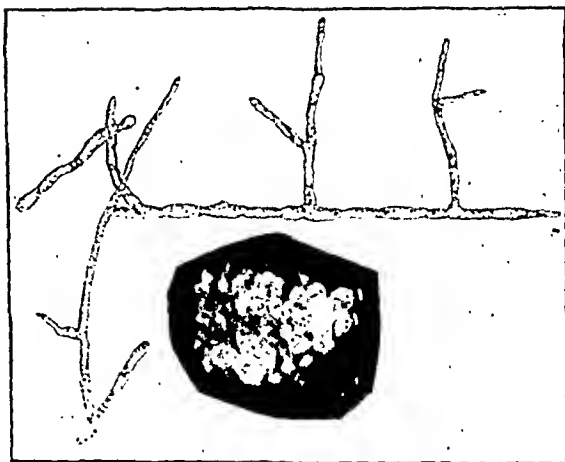
Fig. 31.—*Sterigmatocystis*.35. *Penicillium* Link, 1809.

Sterile hyphae creeping, septate; conidiophores erect, apically irregularly verticillate-penicillately branched; conidia in chains, spherical or elliptical, hyaline or variously colored (Fig. 32).

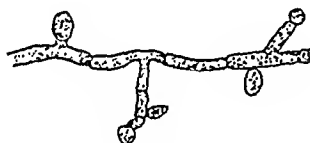
Fig. 32.—*Penicillium*.

36. *Acladium* Link, 1809.

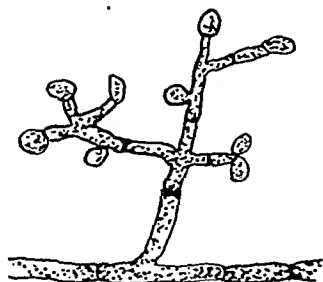
Sterile hyphae creeping, pale, elongate, septate; fertile hyphae but little differentiated; reproduction by simple aleuriospores, growing from the sides of the unbranched sporophores. Fig. 33, organism and colony.

Fig. 33.—*Acladium*.37. *Aleurisma* Link, 1809 (Vuillemin).

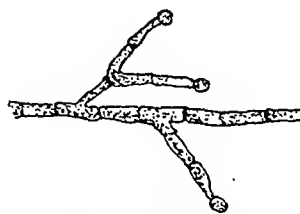
Hyphae branched, pale, elongate, septate; reproduction by simple aleuriospores; true conidiophores absent; sporogenous apparatus well differentiated from the mycelium; spores smooth, small, colored, grow at apex and sides of the sporophore (Fig. 34).

Fig. 34.—*Aleurisma*.38. *Corethrospis* Corda, 1839 (Brumpt).

Hyphae septate, elongate, branched; reproduction by aleuriospores; true conidiophores absent; sporogenous apparatus well differentiated from the mycelium; spores simple, pale, small, smooth, at the tip and on the sides of the sporophore (Fig. 35).

Fig. 35.—*Corethrospis*.39. *Glenospora* Berkley and Curtis, 1876 (Brumpt).

Hyphae pale, dark, septate, branched, elongate; reproduction by aleuriospores; true conidiophores absent; spores simple, becoming dark on light or dark hyphae, situated at tip and sides of sporophore, small, generally 4 by 6 microns, rarely 5 by 11 (Fig. 36).

Fig. 36.—*Glenospora*.

40. *Sporotrichum*  
Link, 1809.

Hyphae hyaline, much branched; septate or continuous; conidiophores simple, short; conidia solitary or in groups on separate sterigmata, ovoid or subglobose. Organism and colony shown in Fig. 37.

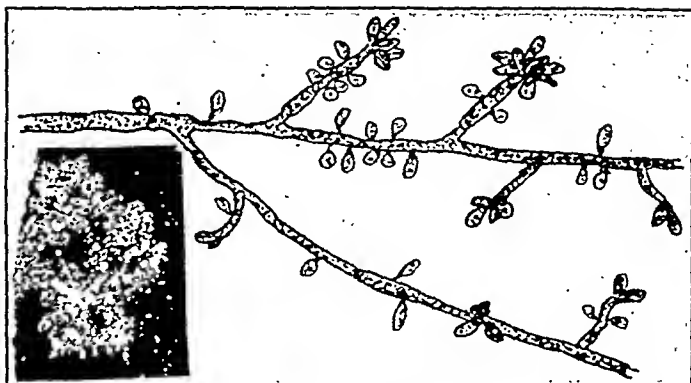


Fig. 37.—*Sporotrichum*.

41. *Mycoderma* Persoon, 1801.

Hyphae septate, tough, diameter greater than 1 micron. Thallus breaks up into spores (arthrospores). Form a scum on liquid mediums and yeast-like cultures on solid mediums (Fig. 38).

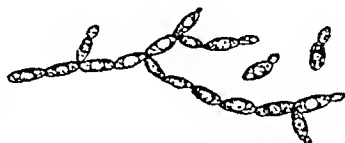


Fig. 38.—*Mycoderma*.

42. *Torula* Persoon, 1801.

Sterile hyphae decumbent; conidiophores short, scarcely different from the conidia; conidia in chains, breaking away singly or in groups; dark to black, smooth or rough, oblong to fusoid (Fig. 39).



Fig. 39.—*Torula*.

43. *Cryptococcus* Kützing, 1833.

Budding forms without filaments; do not produce ascospores; well-developed double contour.

44. *Debaryomyces* Klöcher, 1909.

Yeast-like organisms with the formation of single ascospores which have a warted surface (Fig. 40).

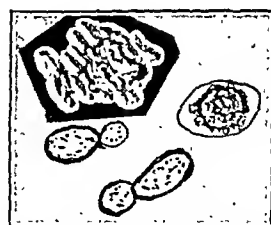


Fig. 40.—*Debaryomyces*.

45. *Saccharomyces* Meyen, 1838.

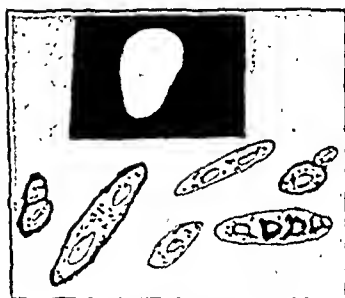
Budding forms with smooth ascospores. Most species form from four to eight spores. Rudimentary mycelium occasionally present. Produce alcoholic fermentation. Fig. 41 shows the budding organisms and an ascus with four spores.



Fig. 41.—*Saccharomyces*.

46. *Willia* Hansen, 1904.

Budding forms with hat-shaped or lemon-shaped ascospores; generally do not produce alcoholic fermentation; various esters produced which give characteristic odors. Form dry, wrinkled pellicles on the surface of liquid mediums. Fig. 42 shows a colony and budding cells with an ascus containing four spores.

FIG. 42.—*Willia*.

## A PHARMACOLOGIC AND THERAPEUTIC STUDY OF BROMSALIZOL, OR MONO-BROM SALIGENIN\*

DAVID I. MACHT, M.D., F.A.C.P., AND FITZGERALD DUNNING, PH.D.,  
BALTIMORE, Md.

### INTRODUCTION

IN 1918, as a result of an extensive and intensive study of opium alkaloids in regard to the relation of their chemical structure to their pharmacologic action, one of the writers announced the discovery of interesting and valuable therapeutic properties exhibited by two chemical compounds. One of these, the ester, benzyl benzoate, exhibited a remarkably relaxant effect on the tonus of smooth muscle organs and an inhibitory action on their peristaltic movements;<sup>1</sup> while the other, benzyl alcohol, was found to be an efficient local anesthetic.<sup>2</sup> In consideration of their low toxicity, clinical observations were made with these two drugs on a selected number of patients; and the favorable therapeutic results obtained soon led to their widespread use in medicine and surgery.<sup>3, 4, 5, 6</sup> When these discoveries had been made public, numerous investigators immediately began a search for analogous or other closely related compounds with the object in view of improving on the therapeutic value of the original substances. Thus, for instance, Hirschfelder<sup>7</sup> pointed out that hydroxy-benzyl alcohol, commonly known as saligenin, which is found in Nature combined with dextrose in the glucoside, salicin, was also a local anesthetic but more stable and more soluble in aqueous solutions than phenmethylool. Again, Barbour<sup>8</sup> and coworkers<sup>9, 10</sup> noted that not only benzyl alcohol, or phenmethylool, possessed a local anesthetic action but that phenethylool and certain other closely related, nonnitrogenous aromatic compounds exerted a similar effect. With regard to the search for a more satisfactory and efficient antispasmodic drug, however, none of the compounds synthesized and tested up to this time has been found to equal in its therapeutic value the oily and rather disagreeably tasting

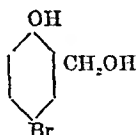
Received for publication, February 23, 1934.

\*Read before the Southern Section, Society for Experimental Biology and Medicine, meeting conjointly with the Southern Medical Association, in Richmond, November 15, 1933.

ester, benzyl benzoate, which has retained its place for fifteen years in the armamentarium of the practicing physician, particularly for the relief of spastic dysmenorrhea and spastic conditions of the gastrointestinal tract. In the past four years, Dr. Fitzgerald Dunning and his associates in chemical research have been engaged in the preparation of a large number of compounds related to the benzyl esters, benzyl alcohol and to saligenin;<sup>11</sup> and these compounds have been subjected to a careful pharmacologic and toxicologic examination. As a result of such cooperation in research between chemist and pharmacologist, a series of compounds has been discovered which exhibit marked sedative or antispasmodic effects on smooth muscle preparations, more powerful than those produced by benzyl benzoate, and which at the same time possess certain other interesting physical and physiologic properties giving promise of possible value in practical therapeutics. These compounds have been briefly described in two short preliminary papers.<sup>12, 13</sup> The most interesting of these is mono-brom hydroxy benzyl alcohol, to which we have given the name of Bromsalizol; and this compound was made the object of a special intensive and extensive pharmacologic examination. Inasmuch as interesting therapeutic observations have already been gathered in this connection, it is deemed desirable to describe the properties of mono-brom saligenin in this place.

#### CHEMISTRY

Bromsalizol is mono-brom hydroxy benzyl alcohol, or mono-brom salicyl alcohol; that is, a halogen derivative of saligenin with the bromine atom in the para-position to the hydroxyl group. Its structural formula is represented as follows:



Analogous halogenated compounds with bromine, chlorine and iodine atoms in other positions have also been prepared and studied by us. Bromsalizol is a beautifully crystalline white powder with a melting point of from 107.5° to 109° C. It possesses a faintly aromatic odor. When applied to the mucous membranes of the mouth or tongue, bromsalizol has first a bitter taste and then produces a sensation of numbness, which is, in fact, a marked local anesthesia effected by even small amounts of the chemical; and in this respect it resembles cocaine and other well-known local anesthetics. Bromsalizol is soluble in water to the extent of 0.5 per cent and a little more readily soluble in hot water. It is readily soluble in alcohol and ether, but only slightly soluble in other common organic solvents. It can also be dissolved in oil. The drug is quite stable in both solid form and solution, and aqueous solutions do not reveal the growth of fungi because of a decided antiseptic action exerted by the compound. Although bromsalizol forms a sodium salt, when examined in this form it loses its characteristic physiologic properties. The free hydroxyl group is apparently necessary for the production of its characteristic physiologic effects.

## TOXICOLOGY

A very extensive and careful study of the physiologic, pharmacologic, and toxicologic properties of bromsalizol has been carried on in our pharmacologic laboratory. Full data on the subject will be published in a more technical journal. The toxicity of this compound was tested on a large variety of living organisms—bacteria, plants, and animals. The toxicity for higher, warm-blooded animals was found to be remarkably low. The figures obtained for the dog, cat, rabbit, guinea pig, rat, mouse, and pigeon are shown in Table I. It will be noted that the lethal intravenous dose for rabbits, cats, and dogs was 250 mg. per kilogram of weight of the animals. Even large doses could be given by mouth. The largest number of toxicologic experiments were made on rabbits. It was found that the minimal lethal dose for rabbits was 0.5 gm. by mouth. We have repeatedly injected 25 c.c. per kilogram of weight of a 0.5 per cent solution of bromsalizol into the ear vein of a rabbit. Such injections had a sedative effect on the central nervous system or, at most, produced a mild stupor, from which the animals invariably recovered. We have also had occasion to administer as much as 5 gm. of the drug in food to a bear cub weighing 15.4 kilograms with no apparent effect. When fatal doses are administered intravenously to the rabbit, cat or dog, death is due primarily to paralysis of the respiratory center. After intravenous injection of massive doses of the drug, a depressant effect on the circulation is noted. After lethal doses have been administered by stomach tube, postmortem examination reveals a congested condition of the gastric and intestinal mucous membranes and a passive congestion of the kidneys.

TABLE I  
TOXICITY OF BROMSALIZOL

ANIMAL	METHOD OF ADMINISTRATION	DOSE PER KILO.	RESULT
Dog	By stomach tube	1.0 gm.	Fatal
Dog	Intravenous injection	0.25 gm.	Fatal
Cat	Intravenous injection	0.25 gm.	Fatal
Rabbit	By mouth	0.5 gm.	Fatal
Rabbit	Intravenous injection	0.25 gm.	Fatal
Rabbit	Intravenous injection	0.1 gm.	Mild stupor; recovery
Pigeon	Injected under wing	0.75 gm.	Fatal
Guinea pig	Intraperitoneal injection	1.0 gm.	Fatal
Guinea pig	Intraperitoneal injection	0.2 gm.	Mild depression; recovery
White rat	Intraperitoneal injection	0.4 gm.	Fatal
White rat	Intraperitoneal injection	0.15 gm.	Coma; recovery
White mouse	Intraperitoneal injection	0.5 gm.	Fatal
White mouse	Intraperitoneal injection	0.2 gm.	Coma; recovery
Cub ( <i>Ursus Americanus</i> )	By mouth	0.5 gm.	No effect

## SALIENT PHARMACOLOGIC PROPERTIES

Two striking pharmacologic properties are exhibited by bromsalizol when used in even very small doses. One of these is its effect on smooth muscle tissue; the other is its action on sensory nerve endings.

*A. Antispasmodic Effect.*—Bromsalizol exerts a characteristic sedative action on living smooth muscle fibers and organs containing them, producing a lower-

ing of the normal tonus of the muscle tissue and an inhibition of its spontaneous normal rhythmic contractions. This can be readily demonstrated on some of the organs in the whole animal *in situ* and can be still more strikingly shown on all smooth muscle organs when studied *in vitro* by means of the surviving muscle strip preparations suspended in warm, oxygenated Locke's solution. The sedative and antispasmodic effect on the intestines can be readily observed in cats, rabbits, rats, and the dog after intravenous or intraperitoneal injections of aqueous solutions, when the abdomen is opened and the viscera are inspected with the naked eye. The relaxation of the intestines may also be observed by direct application of the solution to the serosa, or the outer coat of the intestinal wall. This is followed by a vasodilatation and relaxation of the intestinal loop. We have also demonstrated the inhibition of intestinal movements by special experiments on rats in which the passage of intestinal contents after introduction of the drug by stomach tube is measured by a special method described by one of the authors elsewhere.<sup>14</sup>

The best demonstration, however, of the antispasmodic and sedative or depressant action of small doses of bromsalizol on smooth muscle viscera is afforded by the study of surviving muscle preparations taken from various organs and kept in oxygenated physiologic solutions. In this way, we have performed numerous experiments on smooth muscle preparations from the intestines, uterus, gallbladder, urinary bladder, fallopian tubes, seminal vesicles, ureters, arteries and other organs from the rat, guinea pig, rabbit, cat, dog, bear, and woodchuck.

Fig. 1 illustrates the sedative effect of mono-brom saligenin on an intestinal preparation from the cat; Fig. 2 shows the lowering of the tonus and inhibition of the contractions in the uterus of the guinea pig; and Fig. 3 illustrates the relaxation and inhibition of contractions on a muscle preparation from the fundus of the urinary bladder. Such antispasmodic effects can be produced by even weaker concentrations of bromsalizol than 1:10,000. It will be seen from the illustrations that while the drug inhibits the rhythmic contractions of the organs and relaxes their spasm, this action is not equivalent to a complete paralysis and death of the tissue because the contractions can be started up again by the application of a suitable pharmacologic stimulant such as pilocarpine in case of the intestinal preparation, ergotoxin in case of the uterus, etc. Comparative studies on the relative antispasmodic efficiency of saligenin alone and mono-brom saligenin revealed that the mother substance, saligenin, by itself, possesses but very mild sedative properties for smooth muscle while the brominated derivative is from five to ten times more powerful in this respect.

**B. Anesthetic Effect.**—The other striking pharmacologic property of bromsalizol is its local anesthetic effect on nerve endings and nerve fibers. This local anesthetic action can be crudely detected by *tasting* the drug, when a marked numbness is imparted to the tongue and mucous membranes of the mouth. In order to ascertain more accurately the local anesthetic efficiency of bromsalizol, the writers carried out experiments by the following well-known pharmacodynamic methods:

1. *Instillation of solutions into the conjunctival sac:* Concentrations of from 0.1 to 0.5 per cent of bromsalizol, tested on the rabbit's eye by this method, exhibited a definite local anesthesia, as shown by the absence of corneal reflex.

2. *Studies on frogskin:* Reflex frog preparations were used to study the response of the legs dipped in 0.5 per cent hydrochloric acid before and after application of the drug solution in various concentrations to the skin. Such experiments also revealed that bromsalizol was a powerful local anesthetic. Solutions of 0.5 per cent produced anesthesia lasting half an hour after the frog's leg had been dipped in the drug for two minutes.

3. *Experiments by the wheel method on guinea pigs:* After shaving the hair from the abdomen of the guinea pig, a few drops of a solution are injected intradermally. The reaction of the animal to the pain produced by application of the electrodes attached to a Harvard induction coil is studied first on the

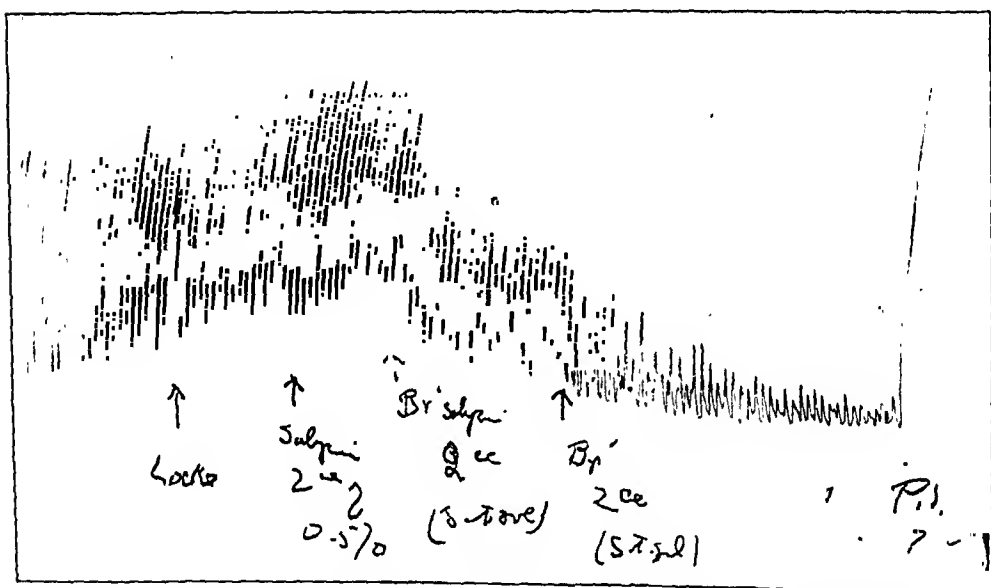


Fig. 1.—Small Intestine of cat. Effect of saligenin, mono-brom saligenin, and pilocarpine hydrochloride.

normal area and then on an area in which the local anesthetic has been injected. It was found that a definite local anesthesia was produced by such intradermal injections of bromsalizol.

4. *Direct application to nerve trunks:* Solutions of the drug were applied directly to nerve fibers; and sensory and motor nerve conduction was blocked, as studied by application of electric stimuli.

5. *Experiments with direct infiltration of bromsalizol solutions in the skin, connective tissue and muscle tissue of higher animals and subsequent surgical operations on such areas:* Such injections produced a satisfactory local anesthesia so that various surgical operations were performed without inflicting pain on the animal.

On comparing the local anesthetic efficiency of mono-brom saligenin with saligen itself, both in regard to the least concentration required to produce



local anesthesia and in regard to its duration when produced by equivalent amounts of the respective drugs, it was found that the brominated compound was four or five times more efficient as a local anesthetic than saligenin alone. It was furthermore established experimentally that no undesirable synergistic

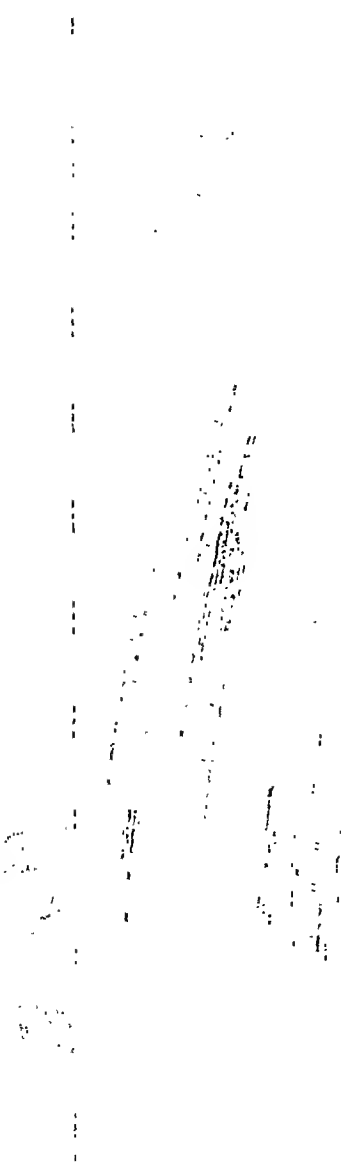


Fig. 2.

Fig. 2.—Uterus of the guinea pig. Relaxation produced by mono-brom saligenin and contraction started up again by ergotoxin phosphate.

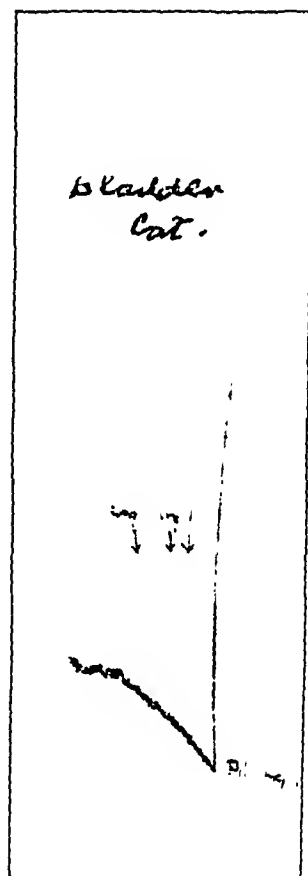


Fig. 3.

Fig. 3.—Fundus of bladder of cat. Relaxation produced by mono-brom saligenin and contraction by subsequent dose of pilocarpine.

effects resulted from combinations of the drug with either epinephrine or ephedrine; and such mixtures effectually abolished the vasodilatation produced by bromsalizol itself by reason of its direct action on the muscle fibers.

## EFFECT ON VITAL FUNCTIONS

An exhaustive pharmacologic investigation of any drug must include a study of its action on all the principal physiologic functions of different animals; and in connection with chemicals offering possibilities of therapeutic use such studies are absolutely essential before even the most cautious tests on clinical material may be undertaken. The authors have been making an intensive and extensive pharmacologic study of bromsalizol, a detailed description of which will be published in a more technical journal. In the present paper only a brief statement regarding the action of this new drug on the most important functions of the living body is given.

*A. Effect on the Central Nervous System.*—Small doses of the drug, comparable to those administered to patients, which will be described further on, were found to have no depressant effect of any kind on lower animals. Such studies were made particularly in psychologic experiments on albino rats trained to run in a circular maze by a method described by one of the writers in various publications.<sup>15, 16</sup> Doses of from 1 to 5 mg. of bromsalizol, administered by stomach or injected intraperitoneally in rats, weighing from 150 to 200 gm. on the average, produced no impairment of their behavior in the maze. Larger doses, 10 mg. or more, however, when injected in such rats, produced a mild sedation. When very large doses are injected into such animals as the guinea pig and rabbit, or given to a dog by stomach, a quieting effect is noted. Such doses, however, are many times (from 10 to 20) greater than those ordinarily used for relieving spastic conditions of internal viscera. The effect of intravenous injections is particularly noteworthy. We have injected as much as 25 c.c. of a 0.5 per cent solution of bromsalizol into the ear vein of a rabbit, and the only effect noted was a mild sedation. The animals remained quiet and were not as active as they were normally. When the effects of such injections were compared with those of saligenin, similarly administered, it was found that the latter produced very little sedation, or none at all, so that the quieting effect of bromsalizol must be chiefly ascribed to the bromine atom in that chemical compound.

*B. Effect on Circulation and Respiration.*—In experiments on rabbits, cats and dogs, tracings were made of the effect of the drug on the blood pressure from the carotid artery and of its effect on the respiration as studied by a method described by one of the authors elsewhere.<sup>17</sup> Intravenous injections of bromsalizol solutions through the saphenous or femoral veins effected the following results: From 5 to 10 mg. per kilogram of weight of the drug in single doses exhibited no effect on either the blood pressure or the respiratory curve. Small doses of from 5 to 10 mg., injected every minute, gradually produced a moderate lowering of the blood pressure without affecting in any way the strength of the heartbeat or the frequency and amplitude of the respiratory movements. Single doses of from 10 to 25 mg. per kilogram of weight of the animal, injected intravenously, produced a sharp fall in blood pressure, which returned to normal within a few minutes. After such injections the respiration was occasionally slightly slowed. Injections of 25 mg. of the drug at one time produced a more marked fall in blood pressure and a slight slowing of the

respiratory rate without interfering with either the force of the heartbeat or the *amplitude* of the respiratory movements. When such large doses as those ranging from 25 to 50 mg. were repeatedly injected, the blood pressure remained at a low level without falling further until nearly the lethal dose had been injected. When the maximum dose of the drug, which led to death of the animals, was injected intravenously, the respiration was paralyzed first and the

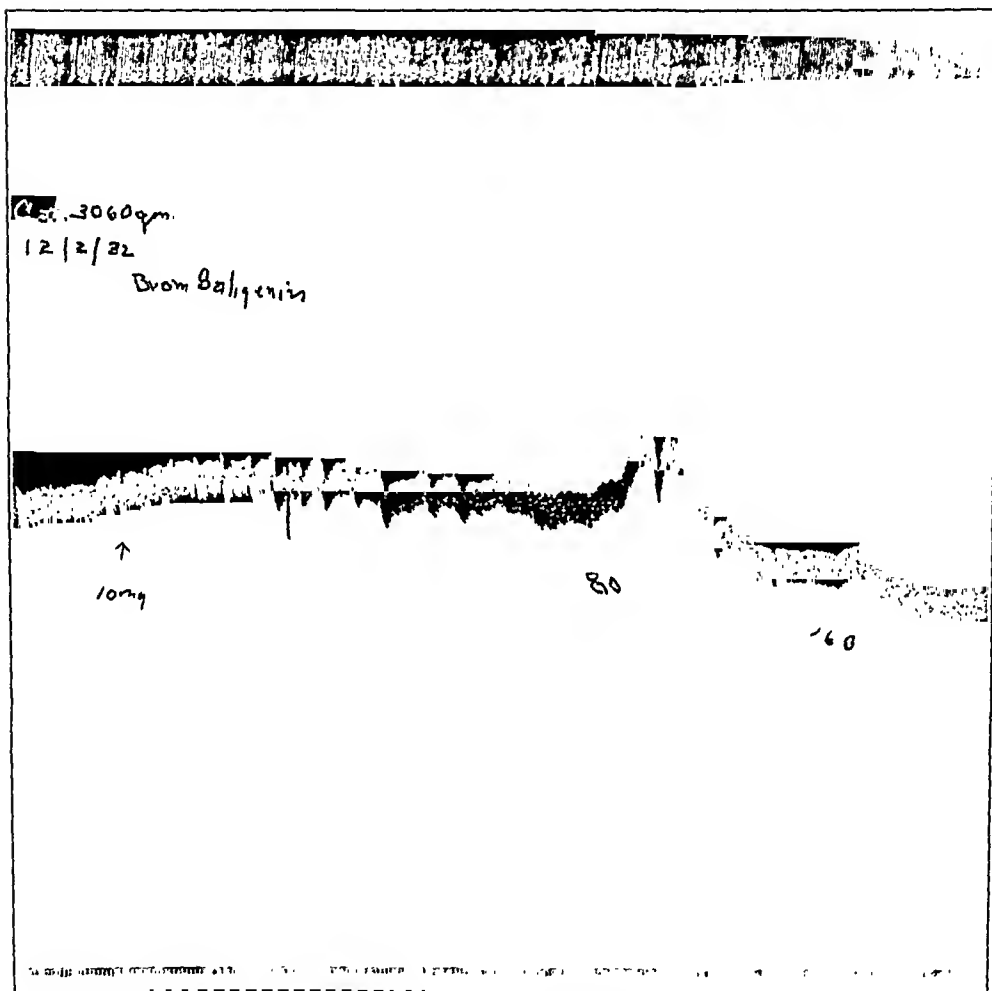


Fig. 4.—Blood pressure experiment on cat under ether anesthesia. The upper is the respiratory curve; the lower, the blood pressure curve.

blood pressure fell gradually after that to a dead level. When such animals were almost dead, they could be resuscitated by the injection of epinephrine. The fall in blood pressure noted after intravenous injections of considerable quantities of bromsalizol is, for the most part, undoubtedly due to the direct peripheral action of the drug on the smooth muscle elements in the arterial walls. This was established by means of perfusion experiments on the frog and by Krafkoff's method of perfusing the rabbit's ear. We have also found that the

drug produces a relaxation of arterial rings or strips suspended in oxygenated Locke's solution, as studied by a technic described by one of the authors elsewhere.<sup>18</sup> Bromsalizol, injected in doses of from 5 to 15 mg., and even more, produces no depressant effect on the vasomotor center and exhibits, if anything, a stimulating action in this respect, because the vasodilatation and fall in blood pressure are much more marked in experiments performed on decerebrated cats. The fall in blood pressure can also be demonstrated more strikingly in vagotomized animals. The effect of the drug on the heart itself was studied on excised hearts of frogs and turtles. Application of 1:10,000 solutions produced little or no effect on the heart muscle. Solutions, 1:1,000, however, when applied directly to the myocardium, either externally or by perfusion through the aorta, gradually depressed the muscle; and concentrations of 1:500 arrested the heart in diastole. Such concentrations, of course, were much greater than those obtained in the blood by administration of even very large quantities of bromsalizol through the stomach.

Briefly summarized, the effect of bromsalizol on the blood pressure and respiration in doses of from 5 to 10 mg. is nil. Slightly larger doses produce a mild lowering of the blood pressure due to peripheral vasodilatation. A depressant action on the circulation and respiration is produced only by excessive and sublethal doses of the drug, in which case paralysis of the respiratory center is followed by depression of the circulation. Fig. 4 shows the effect of monobromsaligenin on the blood pressure and respiratory curves of a cat. It will be seen that 10 mg. of the drug produced no effect on either blood pressure or respiration. Eighty milligrams produced a slightly greater lowering of the blood pressure, and 160 mg. a slightly greater fall in the blood pressure and a slight slowing of the respiration.

*C. Effect on Kidney Function.*—A series of experiments was performed to determine the effect of bromsalizol on the kidney function of rabbits. The drug was administered by stomach tube to some rabbits, intravenously to others, and fed in small divided doses over long periods of time to still other animals. Kidney function was studied by the Rowntree-Geraghty phenolsulphonphthalein test. It was found that unless an enormous and very toxic dose was given to a rabbit no alteration in kidney function was produced. The following records illustrate some of the findings obtained in such experiments:

#### EFFECT OF BROMSALIZOL ON KIDNEY FUNCTION

Rabbit No. 3; weight, 1,740 gm.:

October 13—Phenolsulphonphthalein test; 2-hour output	90%
October 14—Given 250 mg. of bromsalizol by stomach tube	
October 19—Phenolsulphonphthalein test; 2-hour output	85%
October 23—Phenolsulphonphthalein test; 2-hour output	95%

Rabbit No. 4; weight, 1,780 gm.:

October 13—Phenolsulphonphthalein test; 2-hour output	85%
October 14—Given 500 mg. of bromsalizol by stomach tube	
October 19—Phenolsulphonphthalein test; 2-hour output	90%
October 23—Phenolsulphonphthalein test; 2-hour output	90%

*D. Effect on Liver Function.*—Studies were made on the liver function in the rabbit by the Rosenthal bromsulphalein method.<sup>19</sup> Rabbits were daily fed from 5 to 10 mg. of bromsalizol for a week or two, and the liver function was determined at the beginning and at the end of that period. The following protocol shows the findings obtained. It will be noted that no change in liver function was produced by ingestion of bromsalizol.

#### EFFECT OF BROMSALIZOL ON LIVER FUNCTION

Experiment on Rabbit No. 8; weight, 2.2 kilo.:

September 11 Began feeding from 1 to 2 c.c. of 0.5%  
solution of bromsalizol, daily

September 13 Bromsulphalein liver function test:

Reading 3 min. after injection	35%–40%
Reading 15 min. after injection	0%

October 23 Weight of rabbit, 2.14 kilo.

Bromsulphalein liver function test:

Reading 3 min. after injection	35%–40%
Reading 15 min. after injection less than	5%

*E. Effect on Metabolism.*—The effect of bromsalizol feeding on the blood chemistry of rabbits was investigated, and the results obtained are strikingly illustrated by the following protocols. It will be noted that no increase in either the blood sugar or the nonprotein nitrogen of the blood was produced in rabbits fed with bromsalizol.

#### EFFECT OF BROMSALIZOL ON BLOOD CHEMISTRY

Rabbit No. 5; weight, 2 kilo.:

October 9	Nonprotein nitrogen of blood	26.5 mg.
	Blood sugar	105.0 mg.
	Given 500 mg. of bromsalizol in divided doses by stomach tube	

October 16	Nonprotein nitrogen of blood	26.5 mg.
	Blood sugar	100.0 mg.

Rabbit No. 40; weight, 2.2 kilo.:

October 9	Nonprotein nitrogen of blood	36.3 mg.
	Blood sugar	88.4 mg.
	Given 500 mg. of bromsalizol in divided doses by stomach tube	

October 16	Nonprotein nitrogen of blood	29.2 mg.
	Blood sugar	86.9 mg.

#### OBSERVATIONS ON NORMAL MEN

After the observations on animals described above had been completed, we had an opportunity to make some studies regarding the effects of bromsalizol taken by mouth on a number of volunteers about the laboratory. Some of these subjects took only one 5-gr. dose of bromsalizol; others ingested such doses three times; and one individual volunteered to swallow from 10 to 15 gr. of the drug at one time. The men were carefully observed by one of the writers, who

made repeated measurements of their pulse rate, respiratory movements and blood pressure. The results obtained in five individuals are exhibited in the following protocols:

- Subject: P. G. Blood pressure, 129/84. Pulse, 84, and respiration, 20 a minute.  
Given 5 grains of mono-brom saligenin by mouth.  
1½ hours later: Blood pressure, 120/82. Pulse, 88, and respiration, 24 a minute.
- Subject: F. D. Blood pressure, 129/80. Pulse, 72, and respiration, 16 a minute.  
Given 5 grains of mono-brom saligenin by mouth.  
2 hours later: Blood pressure, 124/75. Pulse, 68, and respiration, 16 a minute.
- Subject: M. F. Blood pressure, 124/78. Pulse, 62, and respiration, 18 a minute.  
Given 5 grains of mono-brom saligenin by mouth.  
2 hours later: Blood pressure, 112/70. Pulse, 60, and respiration, 18 a minute.
- Subject: G. S. Blood pressure, 120/74. Pulse, 64, and respiration, 20 a minute.  
Given 5 grains of mono-brom saligenin by mouth.  
2 hours later: Blood pressure, 110/74. Pulse, 64, and respiration, 20 a minute.  
Given 5 grains of mono-brom saligenin again.  
2 hours later: Blood pressure, 104/70. Pulse, 64, and respiration, 24 a minute.
- Subject: A. S. Blood pressure, 144/110. Pulse, 84, and respiration, 18 a minute.  
Given 10 grains of mono-brom saligenin by mouth.  
1½ hours later: Blood pressure, 145/105. Pulse, 96, and respiration, 20 a minute.  
1½ hours later: Blood pressure 142/100. Pulse, 96, and respiration, 20 a minute.

In each case both the systolic and diastolic blood pressure was measured with a Tyco's apparatus. It was found that ingestion of the drug produced practically no change in the pulse rate or in the rate of respiration. Some of the individuals revealed no change in blood pressure; more commonly, however, both the systolic and diastolic blood pressure was lowered a few millimeters. The fall was more noticeable in one individual who had a somewhat higher blood pressure than normal. As to any untoward effects of the drug, especially when taken on an empty stomach, no complaints were made. No gastric irritation was noted but some of the subjects mentioned a feeling of emptiness in the stomach in spite of the ingestion of food after the drug had been taken, an effect which must be ascribed to the local anesthetic properties of bromsalizol. The mild lowering of the blood pressure is undoubtedly due to a peripheral vasodilatation.

#### CLINICAL EXPERIENCES

After having studied the pharmacology of mono-brom saligenin or bromsalizol, and after having established its effective dosage, its maximal tolerated dosage, and its lethal dosage for animals of many kinds, and after having learned that it was quite harmless when given to men who volunteered to take it internally, it was deemed justifiable, and indeed desirable, in consideration of its interesting pharmacodynamic properties as an antispasmodic and as a local anesthetic, to inquire into the possibilities of the drug as a therapeutic agent in carefully controlled clinical cases. In accomplishing this end, we have been fortunate to secure the cooperation of a number of distinguished physicians, who

administered the drug to a series of patients and made careful observations concerning its action. Such clinical cases fall logically into two groups, medical and surgical.

*A. Medical Applications.*—Tablets containing 5 gr. of bromsalizol each were prepared and furnished to physicians for clinical use. The first few experiences revealed that the drug produced some satisfactory results, and the physicians therefore continued to administer bromsalizol to a larger number of patients. The drug was found to be particularly useful in three groups of cases: (1) gastrointestinal, (2) gynecologic, and (3) urologic.

For the first therapeutic tests with mono-brom saligenin, we are especially indebted to Professor Julius Friedenwald of Baltimore and his associates, who made a careful study of its action on a series of gastrointestinal cases. These clinical investigators found the drug efficacious in a variety of gastrointestinal disorders, all of which were characterized by symptoms referable to spastic conditions of smooth muscle organs. Patients were given 5 gr. of the drug three or more times a day. The chemical was well tolerated. There were no signs of gastric irritation after swallowing it even on an empty stomach, and no discomfort or other untoward manifestation was noted. Later, the drug was studied in other cities by a number of other well-known physicians, who also reported similar findings in gastrointestinal conditions. Up to the present time we have received reports on over five hundred clinical cases belonging to this group. The chief conditions in which relief of pain and discomfort were effected by the medicament were: pyloric spasm, spastic conditions associated with ulcers of the stomach and duodenum, gallbladder colic, spastic mucous colitis, and peristaltic unrest of the small intestine. From the pharmacologic point of view, the beneficial therapeutic effects of the drug in these cases must be ascribed in part to its antispasmodic action on smooth muscle and in part to its direct local anesthetic effect on tissues with which the drug comes in contact when introduced into the lumen of the gastrointestinal tract.

The results obtained in cases of spastic dysmenorrhea also revealed that mono-brom saligenin was equally efficient in relieving uterine colic. Here marked relief and even complete abolition of pain have been reported by a number of gynecologists who made a study of the drug. In this connection, it may be well to cite the comment of Dr. R. W. Te Linde, Associate in Gynecology, Johns Hopkins Hospital, one of the first physicians to study the drug, who administered bromsalizol to a series of cases of dysmenorrhea and in his very conservative report stated that fully one-third of the patients had been greatly relieved by the medicament, and added that those relieved had not been relieved by other drugs, such as belladonna and even codeine.

In urologic practice bromsalizol has been found useful in relieving spastic conditions of the bladder, seminal vesicles, and especially in ureteral colic. Several physicians, who reported cases of ureteral colic allayed by administration of the drug by mouth, found that the relaxation of the ureteral spasm facilitated the passage and expulsion of minute calculi. Dr. H. E. van Duzen of Dallas, Texas, has not only been employing bromsalizol by mouth but has also been using it for injection into the deep urethra in the treatment of seminal

vesiculitis and prostatitis. The results which he obtained in a series of cases were so successful therapeutically that he has prepared a paper on the subject.<sup>20</sup>

In addition to gastrointestinal, gynecologic and urologic conditions, in which mono-brom saligenin was found to produce favorable therapeutic results, the drug has been tried by different physicians in various other disorders characterized by spastic conditions of smooth muscle, such as coronary spasm, hypertension due to angiospasm without sclerosis of the vessel walls, a few cases of bronchial spasm, etc. The value of the drug in such cases is still sub judice.

*D. Surgical Applications.*—It has already been stated that mono-brom saligenin was found to be an efficient local anesthetic in laboratory experiments. In consideration of the low toxicity of the drug, it was deemed worth while to investigate its possibilities as a local anesthetic in clinical surgery. Solutions of 0.5 per cent in saline or in water, either with or without epinephrine, were prepared. Such solutions were sterilized by bringing them to a boil. It may be added, however, that bacteriologic tests with mono-brom saligenin showed that solutions of 1:200 were quite antiseptic themselves. Such solutions were furnished to a number of Baltimore surgeons, who employed them in minor surgical operations. We are particularly indebted to two surgeons for scientific cooperation in this connection. Dr. Clarence Colm, associate of Dr. Joseph C. Bloodgood, employed solutions of bromsalizol in minor surgical operations and reported that the drug was equally as effective as novocaine. An even larger series of cases has been studied by Dr. Elliott H. Hutchins at the Mercy Hospital, Baltimore, and in his office practice. He reported that the drug gave complete satisfaction as a local anesthetic in general surgery. In dental surgery, the results obtained were not so clear-cut. Dr. Emanuel Krieger,\* dental surgeon, performed a number of extractions with complete anesthesia after injecting bromsalizol along the nerve sheathes. In other cases, however, he found that the local anesthesia thus obtained was not as effective as that produced by cocaine. Another useful application of bromsalizol as a local anesthetic has been made by Dr. Victor F. Cullen of Maryland State Sanatorium. Dr. Cullen found that strong solutions (from 25 to 40 per cent) of bromsalizol in alcohol, when applied topically to tuberculous nodules and ulcers of the larynx, produced a complete anesthesia, which enabled him to cauterize the affected areas without pain.

#### SUMMARY

The pharmacologic, toxicologic, and therapeutic properties of a new drug, mono-brom saligenin, named Bromsalizol, have been carefully investigated. Two striking pharmacologic properties are exhibited by this chemical—a powerful antispasmodic or relaxant action on smooth muscle fibers and organs containing them, and a definite local anesthetic effect on sensory nerve endings and nerve fibers. These two salient pharmacodynamic effects are produced by doses of the drug far below the toxic concentrations, thus leaving a wide margin of safety for its therapeutic testing on higher animals and man. Doses of from 5 to 10 gr. may be taken three or four times daily with impunity. The pharmacologic effects described above have been substantiated by cautiously conducted

\*Died September 12, 1933.



clinical tests on a selected number of patients, and the results obtained reveal its effectiveness as a therapeutic agent in medical cases characterized by spastic conditions of smooth muscle viscera in gastrointestinal, gynecologic, and urologic disorders, on the one hand, and as a local anesthetic in minor surgery, on the other. The data already collected render a further and more extensive clinical therapeutic investigation of the drug very desirable, and such a study is in progress.

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## THE CLINICAL SIGNIFICANCE OF A VERY LOW CONCENTRATION OF UREA IN THE BLOOD\*

ARNOLD E. OSTERBERG, PH.D., AND NORMAN M. KEITH, M.D., ROCHESTER, MINN.

THE normal range of the concentration of urea in the blood has been accepted as approximately 10 to 50 mg. per cent.<sup>1</sup> The lower values of the normal range have been found in individuals on a low protein intake, a high urine output, or on both. In the last few years one of us (Osterberg) has observed, periodically, in our central clinical laboratory, a blood urea content below 10 mg. per cent. On further investigation of the clinical findings in such a case, the diagnosis was frequently found to be that of chronic wasting disease. In one such case active, chronic diffuse pulmonary tuberculosis was known to have been present, but there was also a complicating renal disturbance. Naturally we were surprised to find that the urea content of the blood in this case was only 6 mg. per cent. These isolated examples of a low urea content of the blood led us to go over the laboratory records for the last four years, and, as a result of the survey, we found 25 cases with widely differing ailments in which the concentration of urea in the blood was less than 10 mg. per cent (Table I). We have been unable to find in the literature a report of a similar series of such low values either in normal individuals or in patients suffering from various diseases.

*Method of Determining Urea.*—For the determination of urea in the blood we have used the van Slyke and Cullen<sup>2</sup> modification of the Marshall urease method on whole, oxalated blood. A preparation of the enzyme of soy bean (arleo-urease) was used and the aeration continued for forty-five minutes. In every instance when values below 10 mg. per cent of urea in the blood were encountered, the determination was repeated several times and always checked.

A chance technical error may be responsible for a low value for urea in the blood in any large series of analyses; however, we believe that these extremely low values for urea are not due to technical error for the following reasons: (1) when a very low value was initially obtained the determination was checked several times; (2) a low value could not be attributed to a decreased activity of the urease enzyme because, simultaneously with these analyses, many analyses employing the same solution of urease were performed on blood with normal and high values for urea, and (3) in Case 1, repeated estimations of urea in the blood over a period of two weeks revealed values of 6, 8, 12, and 10 mg. per cent.

*Clinical Data.*—Several patients were suffering from a serious chronic disease, such as pulmonary tuberculosis, encephalitis, diffuse skin lesions,

\*From the Mayo Clinic.  
Submitted for publication, February 20, 1934.

chronic suppuration, diabetes mellitus, duodenal ulcer, and Addison's disease. In some cases also there were chronic renal lesions, these including bilateral pyelonephritis, bilateral hydronephrosis, tuberculosis of the kidneys, and the diffuse nephritis associated with disseminated lupus erythematosus. The occurrence of a low value for urea in the blood in diabetes insipidus would seem to be a possible result of the enormous water exchange. Similarly, in diabetes mellitus, the low value for urea might be the result of polyuria. In duodenal ulcer with obstruction the water and inorganic metabolism may be markedly upset, but it can readily be corrected by large intravenous injections of fluid. In this condition also a low value for urea in the blood might be the result of a large intake of water. However, in other cases no definite organic lesions or marked physiologic disturbances were demonstrated, and the symptoms were described as being due to nervous exhaustion (see Table I).

TABLE I

TWENTY-FIVE DIFFERENT CASES OF DISEASE WITH CONCENTRATION OF UREA IN THE BLOOD LESS THAN 10 MG. PER CENT

CASE	AGE, YEARS, SEX	WHOLE BLOOD UREA, MG. PER CENT	DIAGNOSIS
1*	24 F	6	Chronic pulmonary tuberculosis, acute pericarditis
2	56 F	8	Chronic pulmonary tuberculosis
3	34 F	8	Tuberculosis of left ovary
4	43 F	8	Left tuberculous kidney, tuberculous cystitis
5	41 F	8	Bilateral hydronephrosis
6	48 F	8	Chronic pyelonephritis
7	68 M	9	Postoperative infection, prostatectomy
8	25 F	6	Disseminated lupus erythematosus, chronic nephritis
9*	28 F	8	Stricture common bile duct, obstructive jaundice
10	25 M	8	Extensive infected burns
11	10 M	8	Lateral sinus thrombosis, pulmonary abscesses
12	44 F	8	Duodenal ulcer, gastroenterostomy
13	63 M	9	Chronic prostatitis and suppuration of pleura
14	30 F	8	Addison's disease
15	31 M	6	Extensive psoriasis
16	39 F	6	Diabetes mellitus
17	16 M	9	Diabetes insipidus, encephalitis
18	44 F	8	Lethargic encephalitis
19	32 F	8	Pregnancy, hypotension
20	64 F	8	Chronic urethritis, obesity
21	20 F	6	Angioneurotic edema
22	44 F	8	Chronic nervous exhaustion
23	57 F	8	Chronic nervous exhaustion
24	45 F	9	Chronic nervous exhaustion
25	58 F	8	Chronic diffuse fibrositis and chronic nervous exhaustion

\*Necropsy.

MaeKay and MacKay<sup>1</sup> have shown that the normal concentration of urea in the blood tends to be distinctly lower in the female than in the male. Their lowest figures, 11 to 20 mg. per cent, always occurred in females. In the present series, nineteen, or 76 per cent, were females, and our lowest concentration, 6 mg. per cent, was found in four females and in only one male. Such findings indicated that a low concentration is more likely to occur in the sick, as well as in the normal, female.

The importance of the effect of a high or low protein, and a large or small water intake on the concentration of urea in the blood is well recognized. In Case 17, the water intake and urine output reached as much as 16,000 c.c. in twenty-four hours. Thus, one might expect a washing out of urea. To a less extent the increased water exchange in diabetes mellitus might have a similar, but less marked effect. In a wasting disease with anorexia and vomiting, there may possibly be a decrease in the formation of urea and a gradual washing out of the urea already present. This is illustrated in Case 1. Repeated estimations of urea in the blood over a period of two weeks revealed values of 6, 8, 12, and 10 mg. per cent. During this period there was a distinct increase in the intake of food. A similar explanation applies to Cases 2 and 9.

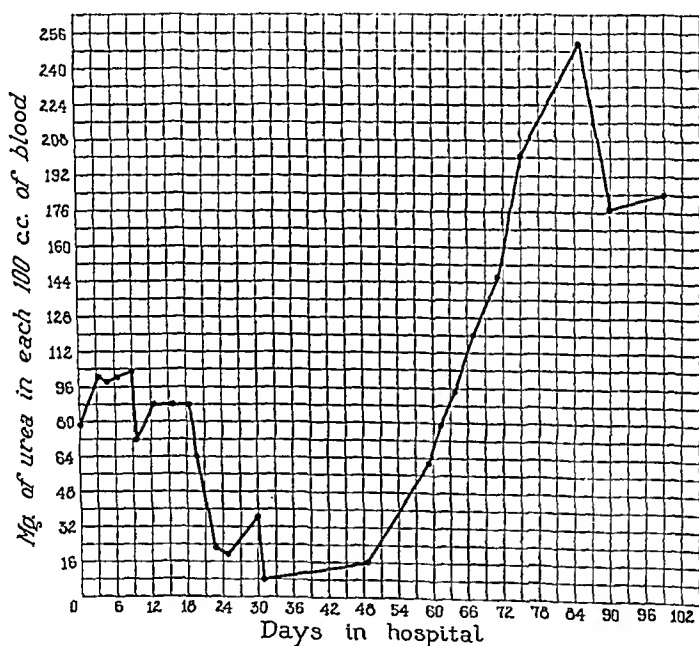


Fig. 1.—Case 8. Disseminated lupus erythematosus, chronic nephritis.

The occurrence of an abnormally low content of urea in the blood in cases with bilateral renal disease seems rather paradoxical. In Case 8, one of disseminated lupus erythematosus, both before and after the very low value for blood urea was found, there was evidence of severe renal insufficiency (Fig. 1). At the period when the value for urea was abnormally low, 6 mg. per cent, the patient was taking in and excreting a considerable volume of water, but the diet was inadequate. The most plausible explanation is that during a temporary process of healing in the kidneys, urea and water were readily excreted and the production in the kidneys, urea and water were readily excreted and the production of urea was decreased. In this connection it is of interest to note that in Case 1, with chronic pulmonary tuberculosis, albuminuria and cylindruria developed during the last month of her illness, that the value for urea in the blood was extremely low, 6 mg.

per cent, and, at necropsy, there were no demonstrable histologic abnormalities in the kidneys. Thus, it is possible to have a very low value for urea in patients with abnormal kidneys, whether the renal disturbance be due to demonstrable histologic change or to pathologic physiology.

#### COMMENT

An abnormally low concentration of urea in the blood can occur in various diseases. Paradoxical as it may seem, it has been observed in bilateral renal disease. The simplest explanation of such a finding is that there may exist a decreased production of urea because of a disturbed nitrogen metabolism, or an enhanced excretion of urea by the kidney due to increased fluid exchange. Both of these factors may be involved.

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### CHOLESTEROL ESTERS AS A MECHANISM OF FAT METABOLISM\*

HENRY PETERSILIE, B.S., NEW YORK, N. Y.

THE following paper is a summary of 67 cases and 93 determinations of cholesterol ester ratios. These cases are taken from the wards of the Bronx Hospital.

The object of this investigation is to determine the significance of the cholesterol ester ratio in diabetes, liver, and kidney diseases.

*Method.*—The total cholesterol was determined on blood plasma, using the Liebermann-Burchard reaction. Bloor's mixture of alcohol and ether was used in the extraction. The esters were determined by combining the free cholesterol with digitonin, evaporating off the alcohol and ether and extracting the esters with petroleum ether. The petroleum ether extract was evaporated and the residual esters were taken up with chloroform; the final determination was the same as for total cholesterol.<sup>1</sup>

*Cholesterol Esters.*—The normal cholesterol of the blood plasma varies in individuals from 150 to 200 mg. per 100 c.c. Bruger and Somach<sup>2</sup> found the cholesterol varied in four normal individuals from 172 to 264 mg. per 100 c.c. The variation in one individual was plus or minus 8 per cent in twenty-four hours.

Bloor<sup>3</sup> states that under abnormal circumstances the cholesterol level may rise or fall, but in health the cholesterol content is fairly constant. Muller<sup>4</sup>

\*From the Laboratories of the Bronx Hospital, New York City.

Received for publication, February 28, 1934.

I wish to acknowledge my indebtedness to Dr. J. Felsen, director of Laboratories and Medical Research, for his suggestions and advice in the preparation of this paper.

says that cholesterol is synthesized in the body and eliminated through the bile and is reabsorbed through the intestines. Cholesterol synthesis is associated with the adrenals, liver, and spleen. Knudsen<sup>5</sup> states that the esters of cholesterol exist as palmitic, stearic, and oleic acid esters. There is a definite balance between free and bound cholesterol in health and under pathologic conditions. The cholesterol as ester is from 46 per cent to 70 per cent of the total. Strobe<sup>6</sup> found from 56 per cent to 80 per cent of the total is normal for esters.

Leites and Goldbitz<sup>7</sup> describe a test meal of 50 c.c. of olive oil. There is a normal rise of from 8 per cent to 44 per cent in the esters during the first seven hours after ingestion of this meal in normal patients. Failure to give this change indicates catarrhal icterus, cholecystitis, and cirrhosis. Table I indicates no marked change in the ester ratio. This may be a good test, but the discomfort of the patient with the aforementioned pathology precludes the use of this test. Most of these patients will refuse oil or fat.

TABLE I

PATIENT INGESTED 50 C.C. OF OLIVE OIL: CHOLESTEROL AND CHOLESTEROL ESTERS IN MILLIGRAMS PER 100 C.C. OF PLASMA

CASE	DATE	CHOL.	ESTERS	PERCENTAGE	TIME AFTER INGESTION
49 E. S. Diabetes	11/20	275	64	23	
	11/27	157	108	68	Fasting
	11/27	136	92	67	4 hr.
	11/27	162	122	73	7 hr.
53 B. V. Diabetes	11/22	227	166	73	Fasting
	11/22	222	156	70	1 hr.
	11/22	227	150	66	2 hr.
56 D. Normal	2/ 4	166	113	67	Fasting
	2/ 4	171	123	72	4 hr.

*Cholesterol Esters in Liver Disease.*—Strobe<sup>6</sup> states that as jaundice of hepatic origin begins, esters decrease from 13 to 43 per cent from the normal. A fall of cholesterol-ester ratio is good evidence of hepatic disease. Adler and Lemmel<sup>8</sup> state that a lowering of the values is parallel with the severity of acute, subchronic yellow atrophy and icterus catarrhalis; and with recovery normal values return. Table II is a series of determinations made on patients with suspected liver involvement.

Case 14 gives lowered ratios of 35 per cent and 57 per cent two weeks later. Cases 59 and 60 give lowered ratios. The cholesterol-ester ratio does not parallel the icteric index in these cases. In Case 14 the ester ratio returns to normal while the icteric index indicates a more severe jaundice at the time of the second determination. All these patients were on a fat-free diet and where the icteric index indicates possible liver damage, the therapy consists of intravenous glucose. Epstein<sup>9</sup> states that the ester values of the blood plasma in acute parenchymatous damage of the liver are diminished or reduced to zero in proportion to the severity of the disease. The partition is normal in atrophic cirrhosis. Two cases of cirrhosis, Table IV, Case 23, and Table II, Case 16, give normal results. In conclusion, the ester ratio is related

TABLE II

CHOLESTEROL AND CHOLESTEROL ESTERS IN MILLIGRAMS PER 100 C.C. OF PLASMA

CASE	CHOL.	ESTERS	PER-CENTAGE	DIAGNOSIS	ICTERIC INDEX
3	402	234	58	Chronic cholecystitis	Normal
5	230	140	61	Acute cholecystitis and catarrhal jaundice	36
	222	140	63		17
	190	110	58		10
10	256	134	52	Chronic cholecystitis and cholelithiasis	17
11	190	123	65	Chronic cholecystitis	9.3
	190	124	65		
13	225	139	62	Acute cholecystitis	
14	285	98	35	Jaundice	94
	266	153	57		112
16	186	119	64	Cirrhosis	10
18	189	108	58	Chronic cholecystitis	6.2
21	188	122	65	Chronic cholecystitis	
	170	154	90		
31	230	170	74		9.8
33	400	275	69	Carcinoma of bile duct	72-180
35	231	143	62	Carcinoma?	32
	250	166	66		80
38	183	145	78	Acute cholecystitis	
40	204	127	62	Cholecystitis	9.1
47	207	144	69	Cholecystitis	7.2
55	177	120	68	Jaundice	46
59	148	65	43	Pneumonia and icterus	84
60	250	85	34	Niemann-Pick's disease	Normal
22	242	140	58	Generalized tuberculosis	

to liver disease, but the absence of fat from the diet and the injection of intravenous glucose seem to interfere with the ratio.

*Cholesterol Esters in Diabetes.*—Bloor<sup>3</sup> quotes a series of abnormal cholesterol-ester ratios in diabetic patients. Table III, Case 49, is the only abnormal cholesterol-ester ratio which the author has been able to find.

Case 49 had not been treated by diet or insulin at the time of the first determination. This same patient one week later, as shown in Table I, gives a normal cholesterol ester ratio after she was advised on her diet. These results correlate well with the work of Best.<sup>13</sup> He showed that dogs with extirpated pancreas on a normal fat diet deposited fat in the liver. If they

TABLE III

CHOLESTEROL AND CHOLESTEROL ESTERS AS MILLIGRAMS PER 100 C.C. OF PLASMA

CASE	CHOL.	ESTERS	PERCENTAGE	DIAGNOSIS
1	222	125	56	Diabetic gangrene
4	217	135	62	Diabetic gangrene
	216	139	64	
19	261	166	60	Diabetes
24	354	241	68	Diabetes and chronic arthritis
	286	178	63	Diabetes and coronary thrombosis
20	255	167	65	
32	244	170	69	Diabetes and cystocele
49	275	64	23	Diabetes, untreated
50	170	104	61	Diabetes
51	207	150	72	Diabetes
52	264	194	73	Diabetes
53	227	167	73	Diabetes
54	215	112	52	Diabetes and uremia

are given insulin or are subjected to a fat-free diet, they do not manifest this fatty deposit in the liver. Chaikoff and Kaplan show a lowered ester ratio in experimental diabetes.<sup>11</sup> It is not uncommon to find fatty deposits in diabetic patients at autopsy. The indications are that insulin, or a fat-free diet, prevent the lowering of the cholesterol-ester ratio and the deposition of fat. The cholesterol-ester ratio, as observed from fatty degeneration of the liver in parenchymatous liver disease and the lowered cholesterol-ester ratio observed in diabetes, point to a close relationship between glycogen, glucose, and cholesterol esters. The carbohydrates necessary for the combustion of fats are lacking in the tissues in diabetic patients. This leads to disturbed fat metabolism. This disturbance manifests itself by the lowered cholesterol-ester ratio.

*Cholesterol Ester in Renal Disease.*—Epstein and Lichtenstein<sup>10</sup> quote 11 cases of nephrosis with increased cholesterol-ester ratios. The cholesterol-esters are from 80 per cent to 90 per cent of the total in 6 cases. Two cases give cholesterol esters of 36 per cent and 38 per cent. One of these gives a 90 per cent ratio twelve days later.

Table IV shows three cases of true nephrosis with abnormal albumin-globulin ratios.

TABLE IV  
CHOLESTEROL AND CHOLESTEROL ESTERS IN MILLIGRAMS PER 100 C.C. OF PLASMA

CASE	CHOL.	ESTERS	PER-CENTAGE	DIAGNOSIS	BLOOD CHEMISTRY
8	375	150	40	Nephritis and nephrosis	N.P.N., 70-200
	275	137	49		Albumin-globulin ratio 3/1 and 1/1
	276	156	56		
23	145	92	64	Uremia	Urea N, 41
26	162	57	35	Acute nephritis	Urea N, 33; A/G, 1/1
29	750	461	62	Nephrosis 1 wk.	A/G/0.3/2.2
30	292	231	79	Nephrosis	A/G/1/2
43	143	83	58	Sepsis	N.P.N./50
48	383	333	84	Nephrosis	A/G/1/1
	345	250	72		
54	215	112	52	Uremia	N.P.N./250
58	130	70	54	Intest. obstruct.	Urea N, 88
44	163	120	73	Nephritis	Urea N, 14
62	114	69	60	Nephritis	N.P.N., 46.9

Case 8 was originally diagnosed as nephritis with a normal albumin-globulin ratio of 3 to 1 and cholesterol-ester ratio of 40 per cent. Two months later the patient showed nephrotic symptoms with an albumin-globulin ratio of 1 to 1, and a normal cholesterol-ester ratio. The N.P.N. rose from 70 to 200 at the time of patient's death. Case 26, that of a child, was diagnosed as acute nephritis, with an albumin-globulin ratio of 1 to 1 and a cholesterol-ester ratio of 35 per cent. Cases 30 and 48 are in agreement with Epstein's and Lichtenstein's findings. The conclusion which may be drawn is that if the nephrosis is of nephritic origin, the cholesterol-ester ratio seems to rise from subnormal to above normal. The initial rise of cholesterol depresses the ester ratio and we find fatty deposits in the kidney, due to the lowered ratio. This phenomenon along with the disturbed protein metabolism may be related to the edema of nephrosis. Calvin and Goldberg<sup>11</sup> state that



the hypercholesterolemia of renal disease is probably the result of a disturbance in fat metabolism accompanying the nephrotic syndrome, rather than the cause or result of edema. The cholesterol content of the blood during edema is higher than normal and may remain high after edema has disappeared. The cholesterol values vary directly with the degree of edema, the changes in cholesterol being preceded by the appearance or disappearance of edema. The cholesterol appears to be mobilized from deposits of fat since the edema is usually associated with emaciation and the blood cholesterol remains high. The fatty deposit in the nephrotic kidney may be compared to the fatty deposit in the liver of acute jaundice, both resulting from a lowered cholesterol-ester ratio.

The accompanying diagram is a simple scheme for showing the relationship between glucose and cholesterol esters in the blood and tissues.

<i>Tissues</i>		<i>Blood</i>		<i>Extraneous</i>	
Neutral Fat	$\rightleftharpoons$	Cholesterol Esters	$\rightleftharpoons$	Fats	
↓					
Glycogen	$\rightleftharpoons$	Glucose	$\rightleftharpoons$	Sugar	

There seems to be an equilibrium between neutral fat and glycogen in the tissues; cholesterol esters and glucose in the blood. The neutral fat is conveyed by means of the cholesterol esters. Sobotka<sup>12</sup> designates the cholesterol esters as a transportation form of fats. A disturbance of tissue glycogen, as in diabetes, would shift the equilibrium of tissue glycogen to blood glucose to glycosuria, which I designate as extraneous, or outside the body. The fat equilibrium shifts from ingested extraneous fat to cholesterol esters to a deposition of neutral fat in the tissues. The cholesterol esters fall below their normal percentage, and deposit the fats in the tissues. Insulin seems to reverse this process, causing a retention of glucose and glycogen. The cholesterol esters increase, drawing fat from the tissues.

In fatty degeneration of the liver, that is, acute or subchronic parenchymatous liver disease, the deposit of fat in the liver (Kupffer cells) causes the retention of bilirubin in the blood. Intravenous glucose shifts the carbohydrate equilibrium to the left and the fat equilibrium to the right. In other words, this phenomenon seems to resemble the fat disturbance in diabetes. It also seems significant that jaundice follows a disease which calls on the reserve glycogen, such as pneumonia and influenza. The injection of glucose seems to mobilize the fat from the liver and the cholesterol-ester ratio returns to normal. The icteric symptoms disappear and the liver condition clears up. In nephrosis the emaciated state of the patient is probably due to low glycogen as well as the loss of protein. The diagram indicates a deposit of neutral fat (in the kidney) and a low cholesterol-ester ratio in the initial stages. The high cholesterol-ester ratio of the later stages represents an attempt on the part of cholesterol to remove the fat from the tissues. Epstein and Lichtenstein<sup>10</sup> describe the appearance of fat bodies and cholesterol esters in the urine of nephrotic patients. This points to a complete shift of the equilibrium to the right in the above diagram.

## CONCLUSION

The cholesterol-ester ratio is abnormal in diabetes and parenchymatous liver disease only under certain conditions of diet and therapy. It is abnormal in nephritis and nephrosis. The ester ratio seems to be closely related to carbohydrate metabolism. A simple diagram illustrating this relationship is shown.

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## PRIMARY CARCINOMA OF THE DUODENUM\*

JAMES R. LISA, M.D., JACOB LEVINE, M.D., AND WILLIAM M. FITZHUGH, M.D.,  
NEW YORK, N. Y.

THE duodenum is one of the rarer sites of primary neoplastic growth but cases are reported with increasing frequency. Several extensive reviews are now available, the recent ones of Eusterman, Berkman, and Swann,<sup>1</sup> Meyer and Rosenberg,<sup>2</sup> and Mateer and Hartman<sup>3</sup> being especially complete and presenting both the clinical and pathologic features.

Since their publications several other cases have been reported. Involving the supraampullary region are two cases of Arisz<sup>4</sup> and one each of Paeetto,<sup>5</sup> Rutishauser,<sup>6</sup> and Bookman.<sup>7</sup> The first case of Arisz is clearly one of cancer developing in chronic ulcer. Rutishauser's case presented multiple tumors originating in Brunner's glands and had peritoneal metastases. The carcinoma in Bookman's case originated in aberrant pancreatic tissue. Rutishauser also reported five periaampullary carcinomas. Forni,<sup>8</sup> Giordano,<sup>9</sup> and Marcus<sup>10</sup> each had one case involving the infraampullary region. Marcus' case is of particular interest. Seven years after the removal of a stenosing adenocarcinoma of the duodenum multiple adenomas of the stomach were discovered at operation. He suggests that the basic condition was an adenomatosis of stomach and duodenum with malignant degeneration in the duodenal region. He could find no other instance in the literature of a case similar to his own.

In the Pathological Department of City Hospital from May 24, 1911, to Dec. 31, 1933, there were 358 cases of carcinoma in 3,956 autopsies. Two were primary carcinomas of the duodenum and are the subject of this report.

### CASE REPORTS

CASE 1.—The patient, a white woman fifty years old, was admitted to the service of Dr. E. P. Shelby because of extreme weakness and night sweats. For one month she had become extremely weak, had lost considerable weight and had had severe night sweats. The most striking feature was the extreme lethargy. There was general muscular atrophy. In the left epigastrium was an ill-defined sense of fullness and resistance. Jaundice was absent. The urine contained a faint trace of albumin. Gastric contents: free HCl 20, combined HCl 36, cellulose, yeast starch and fat. Duodenal drainage was unsuccessful, only bile-tinged gastric contents being obtained. Roentgenology revealed an orthotonic stomach without defects, normal duodenal bulb and high fixation of the second duodenal segment.

Course: The course was characterized by marked weakness, intense lethargy deepening into coma, the development of progressive jaundice, bilirubinuria, severe anemia and a low grade septic fever. Vomiting was never present. The only physical findings of note were increasing size of the liver, edema of the abdominal wall, and terminal bronchopneumonia. Death occurred one month after admission and two months after the onset of the illness.

\*From the Pathological Laboratory, City Hospital, Welfare Island, Department of Hospitals.

Received for publication, February 13, 1934.

Anatomical diagnoses: primary carcinoma of the perampullary portion of the duodenum; acute suppurative cholangitis; chronic interstitial pancreatitis.



Fig. 1.—Case 1. A view of the duodenum opened anteriorly. At the papilla is a small excavated ulcer. The common bile duct is dilated.

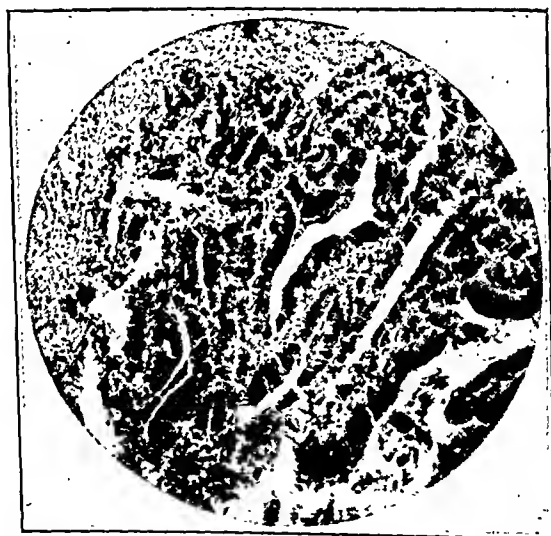


Fig. 2.—Case 1. Photomicrograph of the section through the ulcerated surface, showing the malignant adenomatous structure with marked papillary formation.

The peritoneal cavity contained 200 c.c. of a clear bile-tinged fluid. At the papilla was a small soft friable ulcer 2 cm. in diameter. The duodenum, stomach, common duct and gall-bladder were distended.

*Histology.*—The ulcerated papilla was covered by a necrotic membrane. The edges and adjacent duodenal mucosa had a neoplastic epithelium of high columnar cells characterized by papillary formation. The nuclei were hyperchromatic and mitoses were frequent. Lymphoid reaction was moderate. The lower ends of the pancreatic and bile ducts emptied into the ulcer base; their epithelia were lost by postmortem desquamation but the underlying stroma was normal and free of malignant cells. Metastases were absent.

CASE 2.—The patient, a white male of thirty-four, was admitted to the service of Dr. W. L. Whittemore. One year before he had high substernal pain referred soon after the onset to the eleventh dorsal spine and later to the epigastrium. For a long time it was diffuse and not related to meals. On Christmas a half hour after a hearty meal, he suffered severe epigastric pain relieved by vomiting. Since then similar attacks recurred, the pain radiating

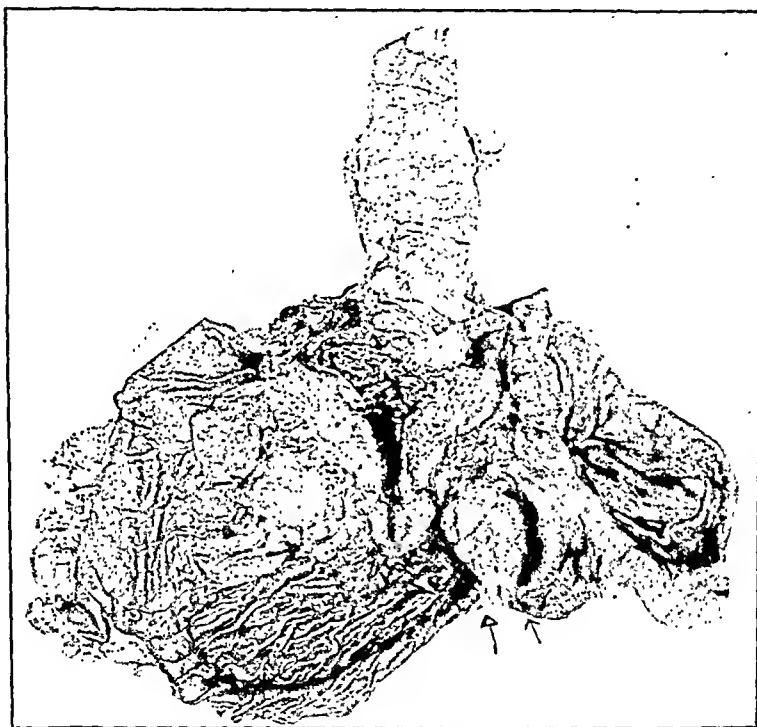


Fig. 3.—Case 2. Gross specimen of duodenum, pyloric region and pancreas. The pylorus is normal. The arrows indicate the upper border, the spear heads the lower border of the carcinoma. The papilla of Vater projects prominently below the lower border. The common bile duct is greatly constricted in its lower portion and dilated above this region.

to the right shoulder. Vomiting and inability to retain any food became the main symptoms. Constipation developed after the onset of illness.

Emaciation was marked. There was an area of tenderness in the epigastrium extending into the right hypochondrium. The liver edge was palpable. Return from an enema was black. Serology and urinalysis were negative. The loss of weight was 30 pounds. Fluoroscopy revealed a dilated atonic stomach filling the entire abdomen and a stenotic duodenal lesion. A test meal could not be done since the slightest irritation caused vomiting.

The course was steadily and rapidly downward. Vomiting was the most prominent symptom; no type of food could be retained. The temperature was septic. Prostration was extreme and death occurred in coma. The entire duration from the onset was approximately one year.

Anatomical diagnoses: primary supraampullary carcinoma of the duodenum; acute suppurative inflammation of the upper retroperitoneal tissues; chronic interstitial pancreatitis; acute bronchopneumonia.

The entire first portion of the duodenum from 1 cm. below the pyloric ring to just above the papilla of Vater was a thick rigid tube with a very narrow lumen. Dense adhesions bound it to the pancreas. The mucosal surface was ulcerated, the cut surface hard and white. The papilla of Vater projected below the lower edge. The lower half of the common bile duct was tightly constricted, barely admitting a filiform bougie; its surface appeared normal. The pancreatic duct could not be probed.

*Histology.*—The ulcerated surface was formed by muscle and covered by a purulent exudate. The entire thickness of the duodenum in this region was infiltrated by neoplastic cells showing marked anaplasia, numerous mitoses and some gland formation. Both edges had a very atypical gland formation, suggesting strongly the glands seen at the edge of chronic

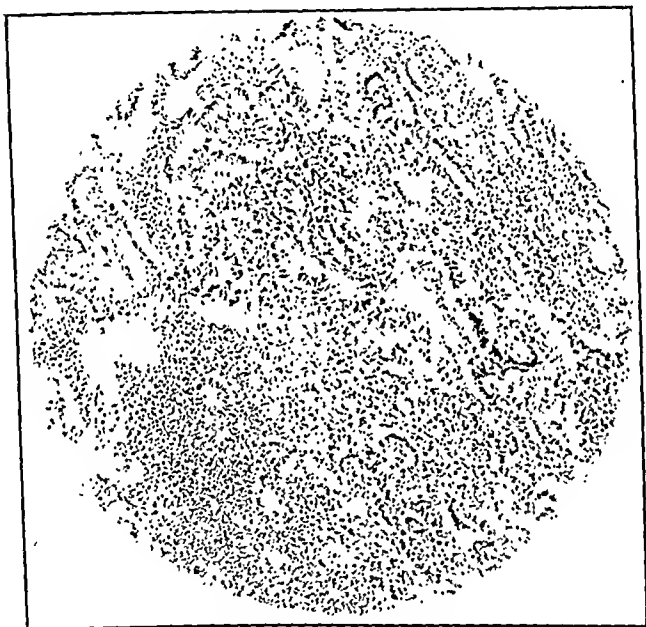


Fig. 4.—Case 2. Photomicrograph of the edge of the carcinoma showing the atypical gland formation.

ulcer. The constricted portion of the common duct was completely encircled by malignancy. The epithelium of duct and papilla was normal. Malignant invasion of the pancreas was present in portions of the perilobular stroma, normal ducts and malignant nests lying side by side in sharp contrast. Miliary abscesses and a diffuse streptococcal inflammation involved the upper duodenum and the retroduodenal and peripancreatic tissues.

#### DISCUSSION

The two cases present many of the characteristic features of duodenal carcinoma. In the first case, the hepatic complication was the predominant factor, a finding which coincides with all cases in which the primary site is the same. The late onset of jaundice suggests strongly that the papilla was involved by extension of a carcinoma originating in the duodenal mucosa of the neighborhood.

In the supraampullary carcinoma the vomiting was greater than usually occurs in pyloric malignancy. Occult blood in the stool and development of constipation associated with intense vomiting are stated to be of great value in differentiating this particular site of malignancy from that of the pylorus. The roentgenologic findings were of the greatest diagnostic importance.

The absence of metastases in both cases well exemplifies their late occurrence in duodenal carcinoma.

#### SUMMARY

The recent literature of duodenal carcinoma is summarized. Two cases are reported, one involving the periampullary, the other the supraampullary portion. Their salient clinical and anatomical features are given.

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# ACCIDENTAL INJURY TO THE BOWEL DURING INTRAPERITONEAL INJECTION\*

GENE H. KISTLER, M.D., UNIVERSITY, ALA.

THE peritoneal route of administration is utilized widely in the laboratory and clinic, especially for supplying fluids in dehydration and by pediatricians as a substitute for the intravenous route. Also important, as Pearcey and Weaver<sup>3</sup> pointed out, is the extensive intraperitoneal use of hypnotics by veterinary surgeons, investigators for anesthesia in laboratory animals, and more recently, for hypnosis in man (McGuire<sup>2</sup> and Thursz<sup>5</sup>). The paucity of evidence of injury to the abdominal viscera recorded during the development of peritoneal administration permits injection with relative impunity, providing a few simple precautions are regarded. Ravenel,<sup>4</sup> however, considered mechanical injury to the bowel the cause of fatal acute generalized peritonitis in a child, following two perforations of the jejunum after intraperitoneal injection of salt solution.

Sporadic erosions of obscure etiology in the bowel of cats and dogs anesthetized by nembutal intraperitoneally were observed recently by A. D. Keller.<sup>†</sup> He suggested that the bowel wall may have been injured by the injection needle or nembutal and demonstrated local tissue damage by this barbiturate. In view of this and the importance of peritoneal absorption, it seemed desirable to accumulate as much data as possible on the occurrence of injury to the bowel by the puncture needle and the changes produced by the more common barbiturate, saline, and glucose solutions when injected into the wall of the stomach and intestine. If injury may occur, precautionary measures should be emphasized. In addition, the accidental injection of a substance into the lumen of the stomach or bowel, instead of the peritoneal cavity, might account for the occasional absence of the usual response to the substance.

## ACCIDENTAL INJURIES TO THE BOWEL

The stomach and bowel of approximately 150 dogs and 100 cats were carefully examined after periods of from ten minutes to three months following routine intraperitoneal injection of hypnotics for anesthesia. The ensuing six lesions found in the bowel were directly attributable to accidental injury:

1. A dog examined twenty minutes after nembutal injection demonstrated a linear dark gray red hematoma, 1 cm. by 0.3 cm., beneath the serosa on the lateral aspect of the large bowel near its midportion (Fig. 1, E). At one end of this was a needle-puncture wound of the serosa from which fresh blood was expressed.

\*From the Department of Physiology and Pharmacology, University of Alabama School of Medicine, University, Alabama.  
Received for publication, February 26, 1934.  
<sup>†</sup>Personal communication.



2. In another dog given nembutal and examined an hour after injection, there was a large, recent intramural hematoma in the distal portion of the jejunum with beginning necrosis of the mucosa.

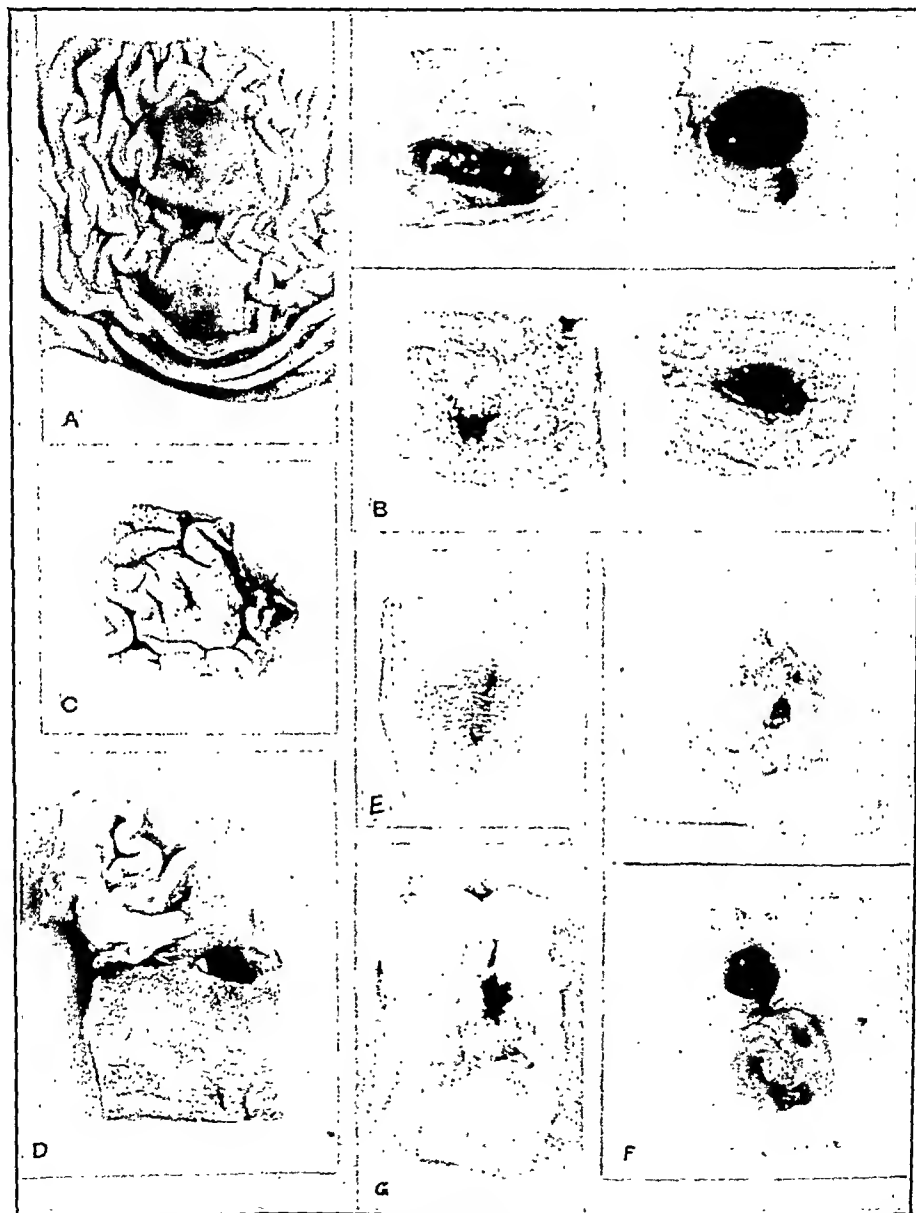


Fig. 1.—Morphologic changes in the stomach and bowel, the result of intramural injections of barbituric acid derivatives. *A*, marked edema and hyperemia of the cardiac portion of the stomach ten minutes after two 0.25 c.c. intramuscular injections of nembutal (1 gr. in 1 c.c.); *B*, erosions of the mucosa opposite subserosal hematomas of the jejunum one and nine hours after subserosal injections of 0.25 c.c. of nembutal (1 gr. in 1 c.c.); *C*, healing ulcer of the cardiac portions of the stomach three days after submucosal injection of 0.25 c.c. of amytal (10 per cent); *D*, large perforation of the duodenum, which occurred on the fifth day after intramuscular injection of 1 c.c. of dial (10 per cent); *E*, *F*, and *G*, injuries to the bowel produced by accidental intramural injections of nembutal (1 gr. in 1 c.c.) during routine intraperitoneal administration of this barbiturate for anesthesia (see text).

3. Six hours after receiving avertin, one dog demonstrated a circumscribed gray blue region in the serosa of the large bowel, 1.2 cm. in diameter, 10 cm. distal to the ileocecal valve. In the center there was a needle hole in the serosa, and the mucosa corresponding to the region was gray red and scraped away easily.

4. Dog 56-B was anesthetized by nembutal intraperitoneally five days before he was killed. There was a healing punched-out ulcer of the upper portion of the ileum (Fig. 1, *F*), 0.7 by 0.3 by 0.3 cm., with a moderately indurated and elevated margin from 2 to 4 mm. wide. Outside the defect in the lining was a gray pink organizing subserosal hematoma, 1.6 cm. in diameter, elevated 0.3 cm., the center of which was depressed and perforation of the ulcer had been prevented alone by the muscularis mucosae (checked by histologic examination).

5. Cat 63 died of acute generalized peritonitis, following a large perforation of the ileum (Fig. 1, *G*), 12 cm. proximal to the cecum, eight days after intraperitoneal nembutal for anesthesia.

6. Dog 67 was examined thirty-five days after receiving nembutal intraperitoneally. There was a stellate scar in the mesentery of the small bowel, 9 cm. from the cecum, that held in close apposition the adjacent serosal surfaces of a loop of ileum.

*Experimental Procedure.*—Through a small midline laparotomy incision under ether anesthesia and aseptic precautions, 155 subserosal, intramucosal, and submucosal injections of from 0.25 to 1 c.c. of the following substances were made with a sharp 26-gauge needle into the wall of the stomach and various levels of the small bowel of dogs: nembutal (pentobarbital, Abbott), 1 gr. in 1 c.c., as recommended for intraperitoneal use; nembutal, made up 1 gr. in 1 c.c. of sterile distilled water; dial (diallyl-barbituric acid, Ciba), 10 per cent with 40 per cent urethane and monoethylurea, as supplied for parenteral administration; 10 per cent solutions of sodium barbital and amytal; 5 and 50 per cent glucose; 0.9, 2, and 30 per cent sodium chloride solutions and sterile distilled water. The viscera were manipulated gently without clamps and multiple needle-puncture wounds were made into and through the wall without injection. The animals were killed and examined immediately after periods of from ten minutes to fifteen days.

*Results.*—The changes produced by the barbiturates varied according to the amount injected, the time elapsed between injection and examination, and according to the position of the substance within the wall. Those produced by dial were slightly more marked than the other derivatives studied. There were no apparent differences in degree of injury produced or healing processes among the stomach and various levels of the small bowel. Within a few minutes after injection (Fig. 1, *A* and *B*), the mucosa covering the thickened portion of the wall was hyperemic, thin, and soft. The vesicles formed by subserosal injections became hemorrhagic in about ten minutes, and the regions of necrosis in the lining over these were larger, but less marked, than those following submucosal and intramucosal injections. With-

in from twenty-four to forty-eight hours there were definite punched-out defects in the mucosa varying in size from a few millimeters (0.25 c.c.) to 1.5 cm. (1 c.c. injection) in diameter. The smaller ulcers were of the lining while the larger frequently extended as far as the serosa. One of these in the duodenum near the pyloric ring, produced by 1 c.c. of dial, perforated on the fifth day (Fig. 1, *E*). There was considerable edema and slight hyperemia in the zone of reaction about the recent ulcers and moderate induration during healing, which began in two or three days (Fig. 1, *C*) and was usually complete without scar of the lining, except following large ulcers, within eight to fifteen days. The subserosal hematomas organized and contracted and were ultimately absorbed. When produced by the larger subserosal injections, their centers occasionally became soft, and they healed with small scars of the serosa.

Distilled water, 0.9 per cent sodium chloride, and 5 per cent glucose produced thickening and slight hyperemia of the wall, which persisted but a few hours without noteworthy change of the lining. Small defects of the mucosa occasionally followed the larger injections of 2 per cent sodium chloride and 50 per cent glucose. The changes produced by 30 per cent sodium chloride were very similar to those of the barbiturates. Needle-puncture wounds of the wall, without injection, and punctures into the lumen were entirely healed and could not be discerned two days after operation.

Histologically, the early changes were characterized by extravasation of blood and partial or complete necrosis of the tissues at the site of injection. The muscularis mucosae retained its structure, unless a large intramucosal injection had been made, and evidently prevented more frequent penetration of the ulcers. During the development of an ulcer, there was little or no noteworthy reaction about the zone of necrosis. After two or three days, the extravasated blood began organization; fibroblastic tissue, slightly infiltrated with round cells, extended into it from all sides and regeneration of the mucosa had begun from the periphery of the lining defect. In five or six days there remained, as evidence of slight injuries, only small contracted, rather loose fibrous regions in the muscularis, over which the lining was narrow and devoid of folds. After small subserosal injections, organizing hematomas alone indicated the sites of injection. Tissue defects extending through the muscularis mucosae healed by granulation and ultimately produced small puckered scars of the mucosa.

#### DISCUSSION

During routine peritoneal administration to animals, injections often are made without due precaution and thorough postmortem examination is usually confined to parts significant only to a particular experiment. In view of this and the marked tendency for gastrointestinal lesions to heal, it seems possible that injury to the stomach and bowel or intraintestinal injection may occur more frequently than is generally appreciated. The same may be true in man during peritoneal administration of fluids and drugs, as pointed out by

Ravenel. More frequent serious injury to the bowel, after its wall has been injected, is probably prevented by loss of a portion of the injected substance through the wound in the serosa. It is significant that four of the six instances of inadvertent injury to the bowel recorded here occurred in the more fixed portion, where it is not free to shift position.

The lesions described here as being due to accidental injury to the bowel are not coincidental, in view of Ivy's<sup>1</sup> conclusion that gastrointestinal changes of any consequence occur rarely, if at all, in the dog. The degree of organization in the hematomas produced by the accidental injury to the bowel was proportionate to the time elapsed since intraperitoneal injection, and the more recent had needle-puncture wounds of the serosa.

The changes in the stomach and bowel following intramural injection of barbiturates may be due to alkalinity and slow absorption. The compact structure of the walls, however, probably plays a part by preventing dissemination. In the concentrations used, the hypnotics were isotonic with from 1.58 to 1.97 per cent sodium chloride. The freezing point of the dial solution could not be determined because of its association with other substances. These equivalent concentrations of salt solutions were slowly absorbed when injected yet produced only slight changes compared to those of the hypnotics and no changes of the lining suggesting necrosis by pressure.

Intraperitoneal injections should be made through a short-beveled medium-sized needle equipped with a stylus. The abdominal wall should be relaxed so that it may be picked up between the thumb and index finger and the thrust of the needle made obliquely to the plane of the wall. After penetration, the needle may be withdrawn slightly to insure freedom of its tip. Particular care should be exercised if the stomach or bowel is distended.

#### SUMMARY

1. Six accidental injuries to the bowel in cats and dogs following intraperitoneal injection are reported. One of these animals died of acute generalized peritonitis, the result of perforation.

2. The bowel is punctured or intraintestinal injection is made more frequently during intraperitoneal administration than is generally appreciated. Simple needle-puncture wounds of the stomach and small bowel heal without consequence.

3. The barbituric acid hypnotics, when injected into the wall of the stomach and small bowel, rapidly produce necrosis of the lining and subsequent changes of the deeper tissues. These changes are probably due to alkalinity and slow absorption of the substance. Although there is a distinct tendency for the lesions to heal, perforation of the wall may occur.

4. Glucose and sodium chloride solutions, unless concentrated, produce no damage when small amounts are injected into the wall of the stomach or small bowel.

5. It is not intended to discourage intraperitoneal administration but such injections must be made carefully to insure peritoneal absorption and freedom from injury to the gastrointestinal tract.

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 THE EFFECT OF BACTERIA ON INSULIN\*
 

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MELVILLE SAHYUN, PH.D., AND PAUL BEARD, PH.D., STANFORD UNIVERSITY, CALIF.

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A FEW definitely established properties of insulin may be briefly summarized as an introduction to the present investigation:

Insulin in its purest crystalline form behaves like an amphoteric electrolyte, has an isoelectric point about pH 5.3, and shows a light absorption in the ultraviolet at exactly the same place as most proteins. It is coagulable by heat and yields simple amino acids on hydrolysis. Its physiologic activity is destroyed by proteolytic enzymes. According to Sjögren and Svedberg<sup>1</sup> it has a molecular weight of 35,100, comparable to that of a large number of proteins.

While the above properties strongly suggest that insulin is a protein, there is a possibility that the physiologically active principle might be a much smaller molecule adsorbed on crystalline material. There appear certain data not entirely in harmony with our conception of protein behavior. In his studies on crystalline insulin, Scott<sup>2</sup> reports:

"It has been observed by various workers that many diabetics who show localized sensitivity reactions to injections of beef insulin can be given hog insulin and show no such sensitivity reactions. Further, certain patients who show a local reaction to commercial preparations of beef insulin show similar reactions to beef crystalline insulin.<sup>3</sup> If crystalline insulin is a chemical entity, it is surprising that individuals should be hypersensitive to it—a protein substance already present in the body."

Those familiar with bacterial metabolism will recall that certain bacterial species are capable of splitting pure proteins and that others lack this faculty. *Proteus vulgaris* is an example of the former and *Escherichia coli* of the latter type. The present investigation endeavors to utilize these phenomena in further understanding the nature of insulin.

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\*From the Department of Bacteriology and Experimental Pathology, Stanford University, California.

Received for publication, February 24, 1934.

This investigation was supported in part by a grant from Eli Lilly and Company, Indianapolis, Ind.

Before entering into details about the experimental work a few words might be necessary regarding the following:

*Organisms.*—Pure cultures of *Proteus vulgaris* and *E. coli* (K-12) were employed.

*Medium.*—Several media were investigated, and the one finally selected to yield suitable results contains the following ingredients:

M/5 Potassium acid phosphate	50.0 c.c.
M/5 Sodium hydroxide	23.6 c.c.
Sodium chloride	5.0 gm.
Glucose	2.0 gm.
0.5 c.c. of a 1 per cent solution of the chlorides of magnesium, calcium, and iron.	

The above ingredients were dissolved and made up to a volume of 1 liter in a volumetric flask by the addition of distilled water that had been boiled and cooled to room temperature. If the ingredients are accurately measured the final pH is exactly 6.8.

*Sterilization.*—This was performed by filtration through a Chamberland candle L 3 and not by autoclaving.

*Insulin.*—Several preparations were investigated, but the majority of the experiments were performed on Lilly's product U-100 and on a sample of crystalline insulin secured from Abel's laboratory.

*Test for Insulin.*—The physiologic activity of insulin was determined roughly on rabbits. The strength of the crystalline material was considered to be 25 units per 1 mg. of the dry material. The tests that were carried out in this laboratory were performed simply to gain a rough estimate of the physiologic activity of the material subjected to the action of bacteria.

*Procedure.*—In performing our experiments great care was taken to insure sterility before inoculation. The medium is first introduced into sterile flasks, followed by the desired amount of insulin, and then inoculation. The flasks were then carefully rotated to obtain a thorough mixture of the ingredients. Samples were removed by means of sterile pipettes for the following tests: pH, sugar determination, bacterial count, and the physiologic assay of insulin introduced. Samples removed after one or more days of incubation (for the physiologic assay of insulin only) were treated with 2 or 3 drops of a 5 per cent phenol solution and allowed to stand for fifteen minutes at room temperature before injecting them into the animals. In this connection it is important to point out that the removal of bacteria by filtration through a Chamberland candle L 3 is not suitable, for the amount of insulin employed is small to begin with, and it was found by preliminary experiments that in such small amounts a considerable loss occurs. Apparently it is adsorbed on the candle.

*Temperature.*—In our earlier experiments incubation of the samples was carried out at a temperature of 37° C. This permitted a rapid growth of the organisms. This temperature, however, was not found suitable for our pur-

poses as insulin without bacteria incubated in weak solutions at 37° C. was found to lose its physiologic activity very rapidly.

*Experimental.*—Into two flasks containing 50 c.c. of the medium, 1 c.c. of U-100 was introduced into each. After three days' incubation at 37° C., 3 c.c. samples were removed of each flask and injected into two rabbits, respectively. The results are given in Table I.

TABLE I

	MG. BLOOD SUGAR PER 100 C.C.	
	INITIAL	ONE HOUR
Rabbit No. 1	119	105
Rabbit No. 2	103	100

*Hydrogen Ion Concentration.*—The most suitable pH of the medium was also investigated. Media containing the same ingredients as described earlier but varying in pH values from 6 to 6.8 were tried. The one most suitable for our purposes was found to be pH 6.8. As an illustration the following data are reported:

Each sample contained 25 c.c. of the medium and 60 units of insulin. The number of bacteria introduced into each was 16,000 per 1 c.c.

SAMPLE	pH	NUMBER OF BACTERIA AFTER THREE DAYS' INCUBATION AT 20° C.
1	6.0	45,000,000
2	6.2	50,000,000
3	6.4	80,000,000
4	6.8	84,000,000

*Action of E. Coli (K-12) on Insulin.*—In the following experiment both crystalline and amorphous insulin were used. Incubation was carried out in an incubator regulated at 20° C. The duration of the experiment was thirty-one days. In repeating this experiment for confirmation of the results reported in Table I, the time was cut down to ten days and fourteen days, respectively. This was necessary as the crystalline material at hand was very limited. The volume of the medium in the flasks was 100 c.c. to which 250 units of Lilly's U-100 and 250 units of crystalline insulin, respectively, were added. This experiment was controlled by inoculating 100 c.c. of the medium containing no added nitrogen with the same number of organisms. With no added nitrogen, it is obvious that any definite increase in numbers must arise from associated impurities in the medium constituents or from some extraneous source, such as atmospheric ammonia.

*Proteus Vulgaris and Insulin.*—Into two flasks containing 100 c.c. of the medium 200 and 100 units of Lilly's insulin were introduced and inoculated with *Proteus vulgaris*. The flasks were then incubated at 20° C. As the number of microorganisms growing from day to day is of relative importance, it is omitted from Table III, but it may be stated that the number introduced was 50,000 per 1 c.c. at the beginning of the experiment, and at the end of the

TABLE II  
 B. COLI AND INSULIN

Each sample contains 250 units of insulin per 100 c.c. of medium. Glucose in the medium is given in terms of mg. per 100 c.c. In performing the assay of insulin, each rabbit was subcutaneously injected with 1 c.c. (2.5 units) per kg. Blood sugar was estimated before and 1.5 hours after the injections.

INSULIN PREPARATION	TIME	MEDIUM			MG. BLOOD SUGAR PER 100 C.C.	
		SUGAR CONCENTRATION	pH	NUMBER OF B. COLI PER 1 C.C.	0 HR.	1.5 HR.
	days	mg.			mg.	mg.
Crystal	0	186	6.7	90,000	95	49
Lilly's	0	187	6.8	90,000	91	65
Control	0		6.8	90,000		
Crystal	1	188	6.7	81,000	88	31 convulsions
Lilly's	1	190	6.8	100,000	95	56
Control	1			100,000		
Crystal	2	190	6.7	500,000	104	30
Lilly's	2	190	6.8	300,000	90	30
Crystal	3	183	6.7	8,000,000	115	53
Lilly's	3	186	6.8	6,000,000	100	51
Control	3			3,000,000		
Crystal	4	185	6.7	5,400,000	85	convulsions
Lilly's	4	180	6.7	7,500,000	87	convulsions
Crystal	5	184	6.7	5,400,000	No test	
Lilly's	5	174	6.7	8,200,000	No test	
Control	5			5,000,000		
Crystal	7		6.6	5,300,000	85	25
Lilly's	7		6.6	80,000,000	80	33
Control	7			3,500,000		
Crystal	8	180			89	convulsions
Lilly's	8	136		200,000,000	87	42
Control	8			3,200,000		
Lilly's	10	110	6.0		90	60
Crystal	12	180	6.6		95	convulsions
Lilly's	12	70	6.0		91	convulsions
Crystal	15			12,500,000		convulsions
Lilly's	15			320,000,000		convulsions
Crystal	22				84*	42
Lilly's	22				92*	52
Crystal	31				90*	40

\*The blood sugar of rabbits that received only 2 units of insulin per kg.

eighth day it grew to 50,000,000. That at the end of the eighth day the physiologic activity of the samples was almost completely destroyed can be seen from the data on blood sugar.

# CONCLUSION

The *Proteus vulgaris* experiment indicates very clearly that this proteolytic microorganism has the faculty of completely destroying the physiologic activity of insulin.



TABLE III

Assay of insulin subjected to the action of *Proteus vulgaris*. Sample No. 1 contains 200 units of insulin and No. 2, 100 units. The amount of insulin administered to the animals was 2.5 units per kg.

SAMPLE NUMBER	INCUBATION PERIOD (DAYS)	SUBCUTANEOUS INJECTIONS (PER KG.)	MG. OF BLOOD SUGAR PER 100 C.C.	
			(0 HOUR) MG.	(1.5 HR.) MG.
1	0	2.5	91	37
1	1	2.5	83	45
1	3	2.5	87	convulsions
2	3	2.5	84	
1	6	2.5	90	convulsions
2	7	2.5	93	
1	8	4.0	87	88
2	8	4.0	91	93

Dealing with a microorganism that cannot attack a pure molecule of protein, the experiment on *E. coli* and insulin justifies the following conclusions:

1. *E. coli* has no effect whatever on the physiologic activity of insulin whether or not impure or crystalline insulin is subjected to its metabolic activities.

2. *E. coli* introduced into a medium in which the only source of nitrogen is derived either from insulin or from the impurities associated with it must derive its nitrogen requirements from the latter and not from the nitrogen of insulin.

3. When crystalline insulin is substituted for the commercial product, the growth of *E. coli* parallels its growth in the medium that does not contain insulin—and in which no other source of nitrogen is available. The slight difference encountered in bacterial count in the crystalline insulin experiment and in the control experiment (eight millions as compared to five millions in the control) may either be due to bacterial variation or to slight impurities associated with the crystals. Such nitrogen impurities adsorbed on the large molecule of insulin might have been derived from the ammonia used in bringing about crystallization (see Abel's method<sup>4</sup>). While such traces of nitrogen may not be noticeable in chemical analytical methods, they are detectable by microorganisms. Consequently, the evidence presented in this investigation supports the theory that insulin is a protein.

Further investigations on the action of other microorganisms on insulin are in progress.

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# THE EFFECT OF MUCIN AND MUCINOIDS ON PEPTIC DIGESTION\*

H. C. BRADLEY, PH.D., AND MILDRED HODGES, M.S., MADISON, WIS.

THE following experimental data may prove of interest to those wishing to evaluate the behavior of gastric mucin and similar substances in terms of properties upon which therapeutic use might be predicated. No attempt will be made to review the extensive literature which has appeared since the original suggestion of Fogelson<sup>1</sup> and of Kim and Ivy<sup>2</sup> in 1931.

Commercial gastric mucin, kindly furnished us by the Wilson Laboratory, loses on dialysis as much as 18 per cent of its solids, of which a third is free tyrosine. A 5 per cent solution dialyzed has a viscosity of about forty times that of water. It is not precipitated by acids and is indeed difficult to precipitate by any of the reagents used for removal of native proteins which

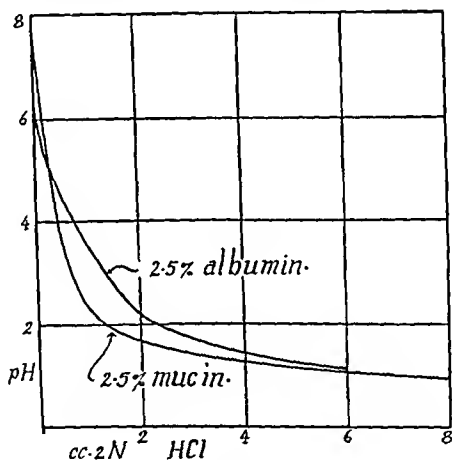


Chart 1.

will at the same time permit the larger cleavage fragments to remain in solution. Alcohol and alcoholic zinc sulphate are fairly satisfactory precipitants. Mucin is not appreciably digested by pepsin as might be anticipated from its history. It has about the same buffer value against acid as any common protein. Chart 1 shows that it is somewhat less effective than egg albumen of the same concentration.

In the following experiments we have studied the effects of mucin on the peptic cleavage of raw fibrin. This protein was selected as the substratum of choice because in the active ulcer, the layer of exudate-fibrin laid down on the surface is believed to be the necessary factor for organization and tissue growth which constitutes healing. Anything which will delay or prevent the digestive removal of this pellicle of fibrin should therefore be of potential

\*From the Department of Physiological Chemistry, University of Wisconsin Medical School, Madison.

Received for publication, February 16, 1934.

value in permitting normal healing to proceed uninterrupted. The figures in Table I and subsequent tables represent the net gain in soluble N in 25 c.c. of the filtrate, or 8.3 c.c. of the digest.

*Fibrin and Mucin.*—Raw fibrin was washed white, ground fine, and pressed free from gross moisture. Twenty grams of this material was used (dry weight 4.2 gm.) suspended in water or 5 per cent mucin, the pH adjusted to the desired level. Five cubic centimeters of 5 per cent pepsin was added, and the digest made up to 200 c.c. and sampled at once. Digestion was carried on at room temperature; the 25 c.c. samples taken were precipitated with 50 c.c. 2.5 per cent  $\text{ZnCl}_2$  in 95 per cent alcohol. Digestion was determined by Kjeldahl's method on 25 c.c. aliquots of the filtrate. Table I shows a typical digestion series.

TABLE I

DIGEST	pH	NET GAIN—C.C. N/5 N IN FILTRATE					
		30 MIN.	1 HR.	2 HR.	5 HR.	1 DAY	3 DAYS
Fibrin + Pepsin HCl	0.6	0.4	0.8	1.4	2.7	6.5	8.3
Fibrin + Pepsin HCl + Mucin	0.6	0.0	0.1	0.3	0.7	3.5	8.0
Fibrin + Pepsin HCl	1.2	1.4	3.2	4.9	5.4	5.4	5.4
Fibrin + Pepsin HCl + Mucin	1.2	0.5	0.9	1.6	2.7	5.1	5.5
Fibrin + Pepsin HCl	2.0	1.4	2.5	3.6	4.8	5.0	5.1
Fibrin + Pepsin HCl + Mucin	2.0	0.5	0.8	1.2	1.9	3.3	3.6
Fibrin + Pepsin HCl	3.0	0.0	0.0	0.1	0.4	0.7	1.6
Fibrin + Pepsin HCl + Mucin	3.0	0.0	0.0	0.2	0.4	0.9	1.5

The precipitation of primary fragments by the alcoholic zinc precipitant is influenced by the acidity of the particular sample taken. The more acid the digest, the more nitrogen remains in solution. The distortion of the series as a whole, however, does not in any way affect the interpretation of data obtained at the same pH levels. Throughout the range studied, from pH 0.3 to pH 3.0, mucin is found to retard the digestion of fibrin. During the first period of from two to five hours it may exceed a 50 per cent reduction. By the end of from one to three days, however, the results are identical. The action of mucin therefore is equivalent to reducing the concentration of the active enzyme. The inhibition is one of rate only. It is most striking where peptic digestion is most rapid. At pH 3 digestion is slow, and mucin has no apparent effect.

*Mucin and Other Proteins.*—The inhibitory effect of mucin is not specific for raw fibrin. We have digested casein, coagulated albumin, fibrin coagulated by heat and by alcohol with and without mucin present. The results are similar to those illustrated above. A typical example is given in Table II.

*Snail Mucin.*—Snail mucin, kindly furnished us by the Burroughs Wellcome Laboratory, has very different properties from the gastric mucin. It is slowly dispersed in slightly alkaline solutions and is precipitated by acids. It is digested by pepsin sufficiently so that interpretation of results is difficult. The enzyme evidently divides itself between the mucin and the fibrin, but, since the digestion of the mixture is not a summation, we may assume some inhibition of fibrin digestion. In view of the flocculation of mucin in acids and its

digestibility, it would appear to be of less value as a protective agent than gastric mucin, which retains its physical properties in acid, and does not digest. (See Table III.)

TABLE II  
CASEIN AND MUCIN

DIGEST	PH	NET GAIN IN C.C. N/5 N IN FILTRATE					
		30 MIN.	1 HR.	2 HR.	5 HR.	1 DAY	3 DAYS
Casein + Pepsin HCl	1.0	5.0	6.2	8.4	12.0	14.8	17.6
Casein + Pepsin HCl + Mucin	1.0	1.1	0.8	2.2	4.2	6.7	10.1
Casein + Pepsin HCl	2.0	2.2	4.5	6.4	8.9	11.5	12.3
Casein + Pepsin HCl + Mucin	2.0	0.3	1.4	2.2	4.2	7.3	8.9
Casein + Pepsin HCl	3.0	1.1	1.7	3.1	3.9	6.7	8.4
Casein + Pepsin HCl + Mucin	3.0	0.0	0.3	0.6	1.4	4.5	7.8

TABLE III  
EFFECT OF SNAIL MUCIN

DIGEST	PH	NET GAIN IN C.C. N/5 N IN FILTRATE					
		30 MIN.	1 HR.	2 HR.	5 HR.	1 DAY	3 DAYS
Fibrin + Pepsin HCl	1.04	0.8	1.5	2.2	3.8	5.6	8.0
Fibrin + Pepsin HCl + Mucin	1.08	0.3	1.0	1.6	2.9	5.7	7.6
Mucin + Pepsin HCl	1.00	0.7	1.0	1.2	--	2.6	3.1
Fibrin + Pepsin HCl	1.95	0.8	1.2	2.0	3.0	4.7	6.6
Fibrin + Pepsin HCl + Mucin	1.94	0.6	1.0	1.5	2.7	6.0	7.3
Mucin + Pepsin HCl	2.0	0.6	0.7	1.0	--	2.4	2.9
Fibrin + Pepsin HCl	3.06	0.2	0.2	0.3	0.7	1.6	2.5
Fibrin + Pepsin HCl + Mucin	3.08	0.4	0.6	0.7	1.0	2.3	3.5
Mucin + Pepsin HCl	3.00	0.5	0.5	0.6	--	1.5	2.1

TABLE IV  
FIBRIN AND CHONDROITIN SULPHATE, 1 PER CENT

SAMPLES DILUTED 1:2 WITH WATER							
DIGEST	PH	NET GAIN IN C.C. N/5 N IN FILTRATE					
		30 MIN.	1 HR.	2 HR.	5 HR.	1 DAY	3 DAYS
Fibrin + Pepsin HCl	0.99	1.8	3.1	4.6	6.5	7.6	7.7
Fibrin + Pepsin HCl + Chond.	1.03	0.0	0.2	0.8	2.0	4.3	5.9
SAMPLES DILUTED 1:2 WITH TRICHLORACETIC ACID							
Fibrin + Pepsin HCl	1.03	0.4	0.8	1.6	3.2	5.6	7.6
Fibrin + Pepsin HCl + Chond.	1.08	0.1	0.3	0.7	1.9	4.7	6.0

TABLE V  
THE EFFECT OF VEGETABLE MUCINOIDS

DIGEST	NET GAIN IN C.C. N/5 N IN FILTRATE				
	PH	1 HR.	5 HR.	1 DAY	3 DAYS
Fibrin + Pepsin HCl	1.0	3.4	8.0	9.9	9.8
Fibrin + Pepsin HCl + Okra	1.0	1.0	5.1	10.9	11.9
Fibrin + Pepsin HCl + Agar	1.0	3.0	6.6	7.9	8.6
Fibrin + Pepsin HCl + Starch	1.0	1.8	5.4	8.9	9.1
Fibrin + Pepsin HCl + Gum Ghatti	1.0	2.3	5.3	10.5	10.4

*Chondroitin Sulphate.*—Babkin<sup>3</sup> has pointed out that gastric mucin may contain some free chondroitin or mucoitin sulphate and that this compound exerts a retarding effect on the digestion of coagulated egg albumen in Mett's

tubes after twenty-four-hour exposure. We have repeated this experiment using raw fibrin and confirm this observation. In this experiment, too, we were able to differentiate between the dispersion of the fibrin, and its cleavage into nonprecipitable fragments. In one digest the samples were precipitated with 5 per cent trichloroacetic acid in water; in the other, the samples were merely diluted. The inhibitory effect of the chondroitin sulphate is much more correctly represented by the digest where no precipitant was used. Solution of fibrin goes on much more rapidly than cleavage to nonprecipitable fragments in the control digests of fibrin alone. The presence of chondroitin sulphate retards dispersion much more than it does further cleavage, as may be seen in Table IV. Dispersion and cleavage in the presence of chondroitin sulphate are practically identical. This fact is of particular significance because it indicates that mucin itself is probably far more potent in delaying solution of fibrin by pepsin than it is in retarding its further cleavage, and from the point of view of therapy this is the important effect.

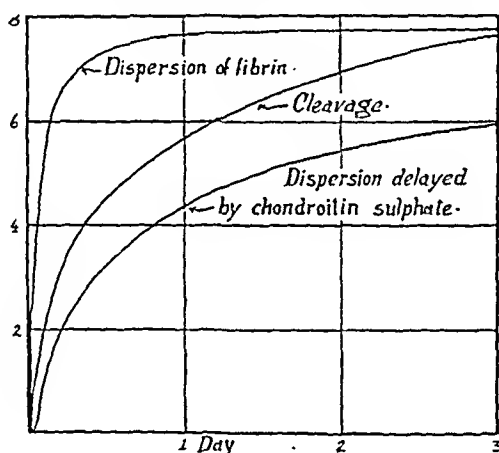


Chart 2.

*Okra, Agar-Agar, Gum Ghatti, and Starch.*—Dispersions of these materials were made to roughly approximate the physical properties of 5 per cent gastric mucin. Five per cent trichloroacetic acid in 95 per cent alcohol was used as precipitant, two volumes to one of the sample. A typical series is given in Table V.

Of these vegetable mucinoids the okra dispersion most nearly resembles mucin in physical properties and in the retardation of peptic activity.

#### SUMMARY

The experiments indicate a temporary delay in the digestive removal of fibrin covered and protected by such mucinous materials as gastric mucin and okra. This inhibition has the same effect as reducing the concentration or activity of pepsin in the digesting mixture. Any delay in the removal of the fibrin pellicle covering the ulcer surface until the stomach contents pass out would appear to be of real significance in preventing interruption of the healing process.

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## THE TOXIC EFFECTS OF SULPHUR ON GUINEA PIGS AND RABBITS\*

GEORGE B. LAWSON, M.D., K. T. REDFIELD, D.V.M., AND OREN D. BOYCE, M.D.,  
ROANOKE, VA.

WE DEMONSTRATED the inhibitory effect of sulphur, even in concentration as small as 1:40,000, on the growth of tubercle bacilli on culture media as a scientific exhibit at the Southern Medical Association Nov. 14 to 17, 1933. As a preliminary to animal experimentation we began a study of the effects of sulphur on guinea pigs and rabbits.

We first did a series of intracardiac injections in guinea pigs and intravenous injections in rabbits with 1 per cent colloidal sulphur in distilled water. (This suspension was not a perfect colloidal solution.) Large doses of this solution were used, ranging from 5 to 10 c.c. both in rabbits and guinea pigs.

There were several fatal reactions at the time of injections, which we thought were due to emboli. These reactions occurred in only three animals. Nine rabbits were used for intravenous injections. These rabbits received 5 c.c. of 1 per cent colloidal sulphur in distilled water per pound of body weight for each injection. Several of the rabbits died at the time of the second injection; but most of them survived four such doses of sulphur and were then killed and autopsied on the fourth and sixth days from the time of the first injection.

The autopsy results varied slightly in minor details, but the almost uniform results were:

1. The lungs showed areas of interstitial pneumonia of a chemical type, without round cell infiltration, but with a few hemorrhagic areas.
2. There were many typical infarctions throughout the lungs and, in some instances, they appeared to be numerous enough to have caused death, whereas in others there were only two or three such areas.
3. The kidneys showed a marked nephritis characterized by marked swelling of the tubules in some and a marked necrosis of the renal cortices in others.
4. The liver sections showed many sulphur crystals throughout the tissue and a few small areas of degeneration.

\*Received for publication, March 29, 1934.

5. Sections of the heart and spleen showed no microscopic changes.

One rabbit received 150 c.c. of 0.031 per cent filtered colloidal sulphur, intravenously over a period of five days. No ill-effects were observed, and he is now living and well, six months after the time of injection.

After intraspinal injections of rabbits with light suspensions of sulphur dissolved in oil (1:500), the rabbits survived and showed no pathologic condition of the cord after twenty-four hours. Injections of a heavy suspension of sulphur in oil (1:55) caused death of the animal in twelve hours. Post-mortem examination showed an edema of the cord and deposits of sulphur crystals around the base of the brain (two rabbits used).

One to two grams of sulphur were injected into the peritoneum of rabbits through a small cannula. It produced a marked necrosis of the abdominal wall with dense adhesions and necrosis of the gut about the point of injection.

Intraperitoneal injections of 10 c.c. of 1 per cent colloidal sulphur in guinea pigs produced a knotting of the omentum incapsulating the sulphur without other adhesions being formed. Injection of 2 c.c. of 1:500 sulphur in oil produced the same result. (Five guinea pigs and four rabbits were used for intraperitoneal injections of sulphur.)

A series of 28 guinea pigs and 2 rabbits were confined to boxes 36 inches long, 18 inches wide and 22 inches deep with open tops. Three or 4 animals were confined to each box and a continuous sulphur duster was installed, delivering a fine sulphur suspension which could only be detected with a strong beam of light. For this we used the commercial three-hundred-mesh sulphur and colloidal sulphur prepared by the Heyden Chemical Corporation. In these experiments the results did not vary markedly with either of the types of sulphur used.

To our surprise, all the rabbits died in from two to four days. The stomach walls were perforated and absolutely necrotic. There was some congestion of the liver, but, this did not seem to be very marked. The guinea pigs all died in from one to three days, and presented the same pathologic picture as did the rabbits. As the material had been blown over the animals as well as over their food and bedding, there was no way to determine accurately just how much of the sulphur had been ingested.

We then tried a series of 41 guinea pigs and 2 rabbits giving doses of from 2 gm. to 0.50 gm. of sulphur per kilogram of body weight, directly into the stomach through a small stomach tube, using in some the colloidal sulphur and in others the flowers of sulphur. To our surprise a similar condition developed, though much more rapidly.

All the animals in this last series died within from two to twenty-four hours. The stomach walls were necrotic, kidneys, intensely congested, and liver slightly so. The spleens showed little change as did the hearts and lungs. This was not a postmortem change, for the same picture was observed when several of the animals were examined under anesthesia.

The kidneys on microscopic sections showed an intense necrosis, almost as though the kidney had come into direct contact with a very powerful

chemical poison. The stomachs were too necrotic to recover more than the connective tissue stroma for microscopic sections.

A possible explanation of these findings was suggested in that sulphur acids may be produced in animals unable to vomit as are the guinea pigs and rabbits. This, however, may have no bearing, but it was thought the very rapid devastating effect of ingested sulphur on the tissues of these animals might be of interest.

#### CONCLUSIONS

Sulphur when ingested by guinea pigs and rabbits produces a necrosis of the stomach and a severe nephritis.



gether by thin threads of chromatin. The distinguishing feature between the segmenters and lobulated stabs is the width of the chromatin joining the lobes. That of the segmenter is never wider than a fine thread. The lobes of the segmenter are oval and have the same cylindrical quality of depth presented by the stab nucleus. The chromatin is compact and may be divided into fairly distinct fields of oxy- and basi-chromatin, which stains dark reddish purple to dark blue. The cytoplasm is the same as that of the myelocyte.

*Description of Hemogram.*—The hemogram is an outline of horizontally arranged figures representing the components of the blood picture. Various combinations signify different conditions, and changes in these combinations from day to day constitute the characteristics by which we may determine improvement, retrogression or complication of the case.

“Schillingizing” the neutrophils, or subdividing them into four classes according to their age and defensive strength determines the quality of the cellular army. With the body at physiologic rest the normal peripheral blood contains no myelocytes, no juveniles, 4 stabs and 64 segmenters; a total of 68 neutrophils in each 100 white cells of all types. An increase in the shift cells, i.e., myelocytes, juveniles and stabs with a corresponding decrease in segmenters is called a “left shift” and means stimulation or irritation. Conversely a decrease in shift cells with a simultaneous increase in segmenters constitutes a “right shift,” and signifies lessened toxicity. The type of left shift is termed degenerative when stabs are increased, regenerative when stabs and juveniles alone, or as many as four myelocytes appear; and leukemoid when stabs, juveniles and five or more myelocytes appear in the hemogram. The type of shift indicates the intensity of the intoxication by its qualitative extent to the left.

The “Schilling index” is the ratio of the combined shift cells to segmenters.

$$M + J + St : Seg$$

$$0 \quad 0 \quad 4 : 64 \quad 4 \text{ to } 64, \text{ or a normal Schilling index of } \frac{1}{16}.$$

This index gives the proportion of inefficient phagocytes to efficient phagocytes. The higher the index, the greater the quantitative shift and the more severe the intoxication.

The “multiple index” is the Schilling index multiplied by 16. Thus the normal is one. It has the same significance as the Schilling index but gives information in whole numbers, showing the number of times a given shift is greater than normal.

The “lethal index” gives the ratio of myelocytes to segmenters, or if no myelocytes are present, the ratio of one-half the juveniles to segmenters. This index gives the proportion of weakest to strongest phagocytes. When the lethal index approaches one, it suggests a diagnosis of septicemia and a prognosis of fatality.\*

\*In attempting to prognose the date and time of expiration we are using the following table with a fair degree of accuracy:

Lethal index 1 and multiple index 100, death in 48 hours or less.

Lethal index 1 and multiple index 200, death in 24 hours or less.

Lethal index 1 and multiple index 300, death in 12 hours or less.

Lethal index 2 or over, regardless of multiple index, death in 12 hours or less.

The "shiftograph" is a vertical graph of the Schilling index arbitrarily adopted by us to express the relationship of the quantitative shift to the qualita-

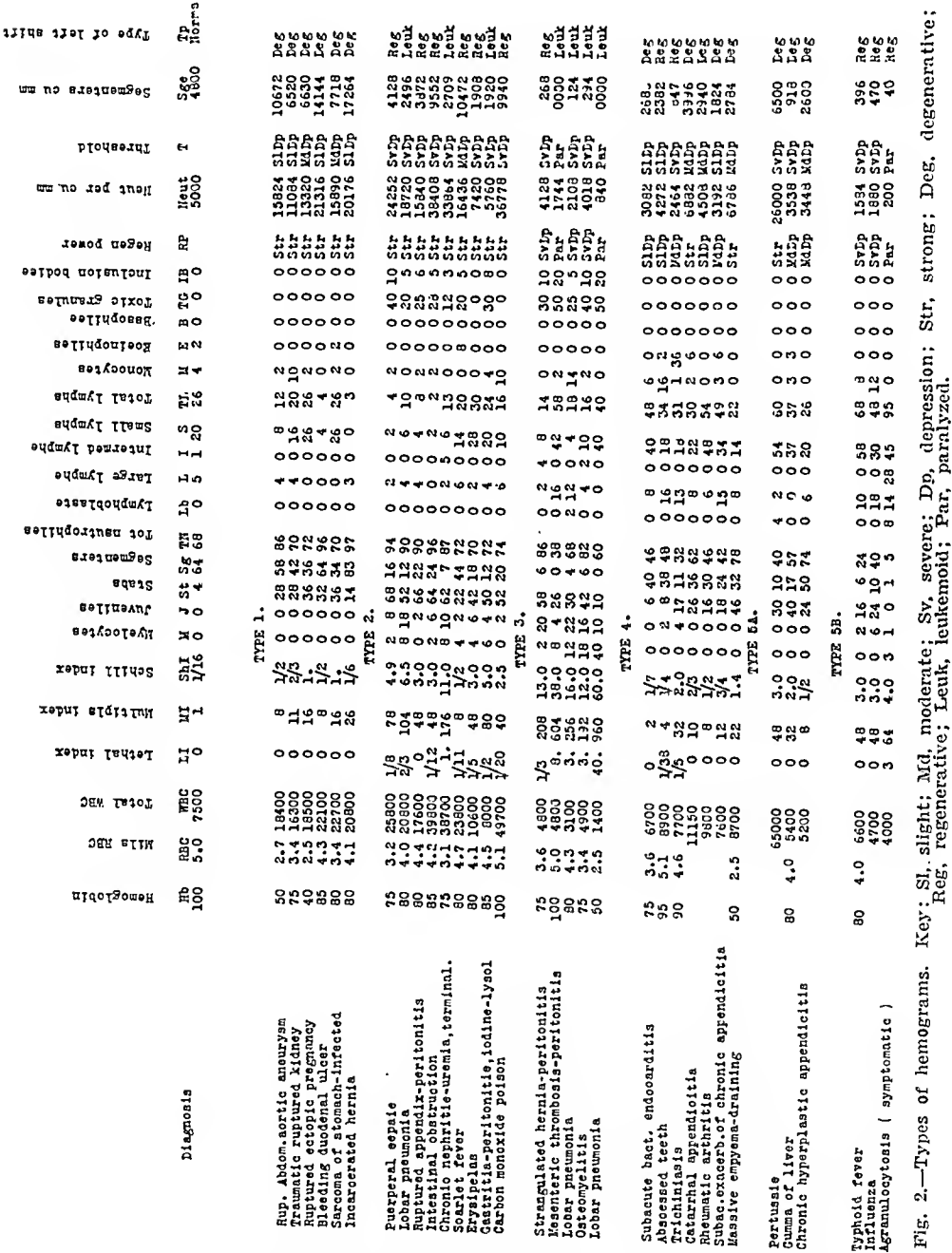


Fig. 2.—Types of hemograms. Key: Sh., slight; Md., moderate; Sv., severe; Dp., depression; Str., strong; Deg., degenerative; Reg., regenerative; Leuk., leukemia; Par., paralysis.

*Reproductive Power "R.P." and Threshold "T."*—It is generally stated that in mild infections maturation of the neutrophiles in the bone marrow keeps pace with the demand for cells, producing a leukoeytosis of the adult type; that in severe infections, although there may be a leukoeytosis, the demand for mature cells is greater than the bone marrow can supply, and immature cells appear in the peripheral blood; while in overwhelming infections either production or maturation or both may break down, producing a regenerative or leukemoid shift with or without leukoeytosis. Again certain irritants or toxins may fail to stimulate the bone marrow or even inhibit production and maturation, then we find leukopenia with immature cells in the peripheral blood.

Whether the factor or factors responsible for these changes in the bone marrow are stimulants or irritants, or whether the changes are the result of positive or negative chemotaxis, we are not prepared to say definitely. For convenience we simply use the term "reproductive power," "RP" of the bone marrow as its capacity for quantitatively producing neutrophiles for the cellular army of defense. It is an index of the reserve strength of the patient and is estimated in general by the total white count and in particular by the percentage of neutrophiles and the number of neutrophiles per cubic millimeter. And we use the term "threshold" "T." as that mechanism which qualitatively controls the maturity of the cells that pass from the bone marrow into the peripheral blood, a normal threshold discharging mature neutrophiles only (64 per cent segmenters and 4 per cent normal stabs), a depressed threshold permitting immature cells to escape, the degree of left shift varying with the degree of depression, and it is with this understanding that these terms will be used throughout this paper in interpreting the size (quantity) and maturity (quality) of the cellular army.

The lymphocytes are Schillingized routinely as the lymphatic shift is very significant. Lymphocytes are divided into four classes: lymphoblasts, large lymphocytes, intermediate lymphocytes, and small lymphocytes. As the large lymphocytes increase at the expense of the small, a left shift is designated, which points to an acute stimulation of the lymphatic tissue or an incipient immunohealing process. A right shift means an increase in the small lymphocytes with a decrease of the large and indicates a chronic stimulation or a definitely established immunohealing phase of repair and convalescence.\*

#### TYPES OF HEMOGRAMS

*Type 1.*—Mild acute stimulation of noninfectious or mildly infectious agents, resulting in a slight left shift.†

\*We realize, of course, that size alone is not an accurate criterion of age of lymphocytes, but this criterion has seemed valid in the shift encountered.

†The one notable exception to the mildly infectious or noninfectious character of conditions which give rise to a Type 1 hemogram is acute suppurative early gangrenous appendicitis with or without rupture of the appendix. The clinical symptoms must be typical of acute appendicitis. The features which characterize this exception are a high total W.B.C. count, 18,000 to 20,000, high neutrophilia, 80 to 100 per cent but only a slight or moderate left shift showing a Schilling index of 1 or less, and a multiple index of 16 or less. When the threshold fails to permit the toxic left shift to be expected with the high white blood cell count, high neutrophilia and typical appendiceal symptoms, we call it a "frozen threshold." Toxin elaborated in the small area of gangrene may be responsible for the inactivity, fixing, or freezing of the threshold.

<i>Blood Picture:</i>	Leukocytosis	10,000 - 20,000	Sh. I.	$\frac{1}{4}$ - 1
	Neutrophilia	80 - 90%	M. I.	4 - 16
	Lymphopenia	5 - 20%	L. I.	0 - $\frac{1}{2}$
	Monopenia	0 - 4%		
	Eosinopenia	0 - 2%		

The reproductive power "RP" is strongly stimulated. The threshold is only slightly depressed. The left shift is slight or moderate, of the degenerative type, sometimes bordering on the regenerative. Noninfectious stimulants producing this picture are, violent exercise, menstruation, pregnancy, and parturition. Noninfectious but severe stimulants are, internal hemorrhage, ruptured ectopic pregnancy, ruptured aortic aneurysm, or ruptured vessel and hernias that are incarcerated but not strangulated. Cysts with twisted peduncles, tumors that are not infected or tumors before necrosis has occurred cause this mild type of shift, later producing a more severe picture.

Infections producing this picture are due to organisms of low virulence or are superficial in nature so that the products of infection are not confined as superficial wounds affecting chiefly the epithelium or draining abscesses. It is also seen in infections affecting mucous membranes with little vascular reaction, as in acute catarrhal cystitis, gastritis, or appendicitis and in the so-called appendicosis.

*Type 2.*—Severe acute irritation, by virulent infections, cytotoxins or poisons, resulting in a marked left shift.

<i>Blood Picture:</i>	Leukocytosis	10,000 - 100,000	Sh. I.	1.5 - 20 plus
	Neutrophilia	90 - 100%	M. I.	20 - 300 plus
	Lymphopenia	0 - 10%	L. I.	$\frac{1}{4}$ - 1 plus
	Monopenia	0 - 4%		
	Eosinopenia	0 - 1%		

"RP" is strong and severely irritated. Neutrophiles 8,000 to 90,000. "T" is strongly depressed and the number of segmenters is reduced; the left shift is regenerative to leukomoid.

This picture is characteristic of many acute infectious diseases, e.g., septicemia, the meningitides, pneumonia, and diphtheria. It is seen in acute suppurative inflammation of mucous and serous membranes with infiltration of the surrounding tissues or with necrosis and gangrene, e.g., in acute suppurative appendicitis, volvulus, intussusception, peritonitis, empyema, abscesses, or confined pus in any location. Certain poisons may produce this type of hemogram, e.g., arsenic, lead, mercury, phenol and its derivatives, and illuminating gas.

*Type 3.*—Lethal acute irritation by overwhelming infections, resulting in an extreme left shift, with a depressed regenerative power.

<i>Blood Picture:</i>	Leukopenia	5,000 - 1,000	Sh. I.	3 - 20 plus
	Neutrophilia	90 - 100%	M. I.	50 - 300 plus
	Lymphopenia	0 - 10%	L. I.	$\frac{1}{4}$ - 1 plus
	Monopenia	0 - 2%		
	Eosinopenia	0 - 1%		

"RP" is inhibited or paralyzed; neutrophiles 5,000 to 500 or less. "T" is severely depressed or paralyzed; the shift is leukomoid in type. This hemo-

gram is produced by extremely virulent acute infection. It is usually seen in septicemia. When it follows a Type 2 hemogram it means bone marrow exhaustion and paralysis with a fatal prognosis. In these cases the total white count is not reliable, as an index of the degree of intoxication, for although the total white count is falling, the left shift is becoming more extreme. Observation of this fact definitely illustrates the fallacy of judging the severity of an infection by the ordinary white count and differential. At the same time it emphasizes the importance of the shift in making a hemogramic prognosis.

*Type 4.*—Subacute stimulations, infections and intoxications, resulting in a moderate left shift, or the lymphomonocytic phase of healing convalescence, the latter following Types 1, 2, and 3.

<i>Blood Picture:</i>	White blood cells	6,000 - 12,000	
	Neutrophiles	50 - 70%	Sh. I. less than one
	Lymphocytes	30 - 50%	M. I. less than 16
	Monocytes	0 - 10%	
	Eosinophiles	1 - 5%	

“RP” is strong, but shows a low grade irritation.

“T” is only slightly depressed. The shift is usually degenerative in type although a few myelocytes and juveniles may occur. The pronounced feature is a moderate to marked lymphocytosis with an occasional slight monocytic increase. This picture is typical of long-continued or lowgrade intoxication. It is also seen transitionally when an acute infection becomes chronic, or when a chronic infection shows a subacute exacerbation. It is common in a subsiding acute infection. As the leukocytosis and neutrophilia fall, the lymphocytes, monocytes, and eosinophiles increase and the shift goes to the right. It is then called the lymphatic or lymphomonocytic immunohealing phase of convalescence.

It is characteristic of lowgrade septicemia, subacute bacterial endocarditis, arthritis, subacute exacerbation of chronic appendicitis; foci of infection, i.e., abscessed teeth, old appendiceal abscess or sinusitis; chronic empyema, pyelitis, draining lung abscess, and degenerating or metastasizing tumors. With an acute flare-up of this type of infection, e.g., extension of suppuration, failure to drain, etc., the blood picture changes to one of the more acute types.

*Type 5.*—Lymphatic type with a moderate left shift. This is caused by acute infections which inhibit or fail to stimulate granulopoiesis and chronic infections which stimulate lymphopoiesis.

<i>Blood Picture:</i>	Normal or leukopenia	10,000 - 1,000	Sh. I. $\frac{1}{2}$ - 2
			(Except pertussis)
	Neutrophilia	50% or below	M. I. 8 - 32
	Lymphocytosis	30% or above	
	Monocytosis	6 - 20%	
	Eosinopenia	0 - 2%	

“RP” is inhibited or not stimulated; neutrophiles average 3,500.

“T” is mildly depressed. The shift is usually degenerative or may border on the regenerative type with a few juveniles or occasional myelocyte. This picture is produced by:

(A) Low grade, chronic toxins which stimulate lymphopoietic tissue, including tuberculosis, syphilis, Basedow's disease and healing processes.

(B) Acute infections which inhibit or fail to stimulate the granulopoietic tissue, including measles, mumps, malaria (latent), influenza, and typhoid fever. It is also seen in subacute or chronic appendicitis, abdominal adhesions, and ovarian cysts. A sudden change from this lymphatic hemogram to a Type 2 or 3, means an acute exacerbation or complication, e.g., secondary pyogenic infection in tuberculosis; onset of pleurisy or pneumonia in influenza; otitis media or bronchopneumonia in measles; perforated ulcer and peritonitis or pyelonephritis, in typhoid fever; orchitis in mumps, etc.

A primary right shift, with a Schilling index of less than  $\frac{1}{16}$  is seen occasionally in pernicious anemia, *Diphyllobothrium latum* infestation, carcinoma with cachexia and anemia, syphilis, pregnancy, malaria, and ankylostomiasis.

#### CASES ILLUSTRATING THE VARIOUS TYPES OF HEMOGRAMS

*Type 1.*—Incarcerated femoral hernia in a white female aged seventy-one years. There was nausea and vomiting, but no other signs of intestinal obstruction. The hemogram was made about eight hours before operation. We were asked whether the hernial contents had become strangulated. At operation a normal loop of gut was released and replaced in the abdominal cavity.

*Hemography:* Shiftogram is a Type 1 hemogram, with high leukocytosis, high neutrophilia, extreme lymphopenia, and a very slight left shift.

*Hemogramic Diagnosis:* Acute noninfectious irritation, incarcerated hernia without strangulation. (Clinically: incarcerated hernia?)

*Hemogramic Prognosis:* Good.

The condition was considered an acute stimulation, rather than an infectious process because of the high leukocytosis with 97 per cent neutrophils, but only a mild degenerative shift to the left. The noninfectious nature of the irritation was shown by such a slight depression of the threshold that the segmenters per cubic millimeter almost equalled the total neutrophils. The slight degenerative shift of 14 stabs was too few to indicate pus; the Schilling index of  $\frac{1}{6}$  was very small and the multiple index only 2.6 times normal. Incarceration without strangulation was indicated by the very slight shift. Strangulation would have shown extreme depression of the threshold, with segmenters less than half the neutrophils and a severe qualitative left shift, including myelocytes and juveniles. The Schilling index would have been greater than one, the multiple index greater than 16, and the lethal index small or large, depending on the severity of infection, gangrene of the intestine, or onset of peritonitis. The prognosis was good because there was no hemogramic evidence of actual injury to the intestines.

*Type 2.*—Lobar pneumonia in a white male, aged forty years. The first hemogram was made on the seventh day of the disease, about six hours before the crisis occurred. Following the crisis there was an apparent relapse, but the temperature again fell by crisis and remained normal to recovery.

*Hemography:* All the hemograms in this shiftogram except the last are Type 2. The hemogramic diagnosis and prognosis are taken from the first hemogram.

*Hemogramic Diagnosis:* Severe acute infection (Clinically: lobar pneumonia).

*Hemogramic Prognosis:* "Bad at present, unless at the time of crisis."

The diagnosis was justified by the extreme leukocytosis, high neutrophilia, leukomoid left shift, high indices, and the presence of Freyfeld's toxic granules and Doehle's inclusion bodies.

A conditional bad prognosis was given on the first hemogram, as it was taken six hours before the crisis (see discussion), and because there were only 6,096 segmenters in a total neutrophilic army of defense of 35,052. Thus, while the defensive force was large, it was of very poor quality. The severity of the infection was also indicated by the high Schilling, multiple, and lethal indices.

In tracing the course of the disease, the shiftograph pointed to a relapse with a wide left shift in Hemogram 3, followed by a continued right shift to convalescence and

Date	Hemoglobin	Mile RBC	Total WBC	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophils	Lymphoblasts	Large lymphs	Intermed lymphs	Small lymphs	Total lymphs	Monocytes	Rosinophiles	Basophilæ	Toxic granules	Inclusion bodies	Regen power	Count per cu mm	Threshold	Segmenters cu mm	Type left shift	No. of hemogram
D	Hb	RBC	WBC	LI	MI	SHI	M	J	St	Seg	TL	Lb	L	I	S	TL	M	E	B	TC	IB	RP	Went	T	Segs	TP	N
80	4.0	20800	0	2.6	1/6	0	0	14	83	97	0	3	0	0	0	3	0	0	0	0	0	0	5000	17264	4800	4800	2
Fig. 3.—Shiftogram of incarcerated hernia illustrating a Type 1 hemogram.																											

Fig. 3.—Shiftogram of incarcerated hernia illustrating a Type 1 hemogram.

Date	Hemoglobin	Mile RBC	Total WBC	Lethal index	Multiple index	Schilling index	Myelocytes	Juveniles	Stabs	Segmenters	Not neutrophils	Lymphoblasts	Large lymphs	Intermed lymphs	Small lymphs	Total lymphs	Monocytes	Kosinophiles	Basophilæ	Toxic granules	Inclusion bodies	Regen power	Count per cu mm	Threshold	Segmenters cu mm	Type of left shift	No. of hemogram
D	Hb	RBC	WBC	LI	MI	SHI	M	J	St	Seg	TL	Lb	L	I	S	TL	M	E	B	TC	IB	RP	Went	T	Segs	TP	N
28	80	4.2	38100	3/4	80	5.0	12	16	48	16	32	0	2	0	4	6	2	0	0	0	0	6	35052	SVTP	6036	Leuk 1	1
29	80	4.2	24800	1/3	61	3.8	6	20	52	19	86	0	4	0	10	14	0	0	0	0	8	8	21328	SVTP	4664	Leuk 2	2
30	80	4.3	20800	2/3	104	6.5	8	8	52	12	90	0	4	0	6	10	0	0	0	0	30	11	18720	SVTP	2896	Leuk 3	3
31	80	4.3	15200	3/4	48	3.0	16	5	39	19	79	0	4	1	13	18	3	0	0	0	20	4	12108	SVTP	2888	Leuk 4	4
1	80	4.3	14800	1/3	16	1.0	2	4	37	36	74	0	16	4	8	24	2	6	0	0	0	0	10352	SVTP	5328	Reg 5	5
2	80	4.3	15100	1/16	16	1.0	2	8	24	32	66	0	14	4	8	26	2	6	0	0	0	0	9366	MDP	4332	Reg 6	6
4	85	4.4	10500	1/24	8	1/2	0	4	22	78	74	0	2	4	20	36	0	0	0	0	0	0	7770	SLTP	5040	Reg 7	7
Fig. 4.—Shiftogram of lobar pneumonia illustrating Type 2 hemograms: 1, 2, 3, 4, 5, 6 are Type 2; 7 is Type 4.																											

Fig. 4.—Shiftogram of lobar pneumonia illustrating Type 2 hemograms: 1, 2, 3, 4, 5, 6 are Type 2; 7 is Type 4.

recovery. The first attempt to enter the lymphatic phase of immunohealing was made in Hemogram 5, with a fall in neutrophils, a rise in lymphocytes, and the disappearance of toxic granules and inclusion bodies. Full convalescence was shown by Hemogram 7, a Type 4, with a lymphatic right shift to small lymphocytes, a neutrophilic right shift to a multiple index of eight, and a high normal total white count.

*Type 2.*—Sepsis of unknown origin. The first hemogram was recorded twenty-five days following the onset of illness. Three weeks later, acting on a hemogramic diagnosis of confined pus, and the clinical evidence of continued sepsis and slightly enlarged tender liver,

a tentative diagnosis of subdiaphragmatic abscess was made and the abdomen opened. A subhepatic or duodenal abscess was suspected but complete exploration could not be done on account of the weakened condition of the patient. Death occurred forty-eight hours later.

*Hemography:* All the hemograms of this shiftogram are Type 2, the neutrophilic phase of defense. Diagnosis, prognosis, and treatment were suggested by the first hemogram and confirmed by subsequent blood pictures.

Date	Hb	HbC	WBC	LI	MI	SHI	MI	SHI	Schill index	Myelocytes	Juveniles	Stabs	Segmenters	Not neutrophils	Lymphoblasts	Large lymphs	Intermed lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Regen power	Neut per cu. mm.	Threshold	Segmenters cu. mm.	Type of left shift	No. of hemogram
22	75	3.0	17000	1/3	35	2.2	0	15	40	25	80	5	4	1	10	20	20	10	10	0	0	0	0	0	0	Str	13500 SVLP	4250 SVLP	2800 Reg	1
23	74	3.0	14000	4/5	40	2.5	0	32	20	72	80	4	4	0	20	20	10	20	10	0	0	0	0	0	Str	10080 SVDP	2800 Reg	2		
24	74	3.0	15900	4/5	48	3.0	0	27	20	80	80	4	4	0	20	20	10	20	10	0	0	0	0	0	Str	12770 SVDP	3180 Reg	3		
25	74	3.0	13300	1/5	28	8.0	0	22	30	10	92	0	0	0	6	0	0	6	2	0	0	0	0	0	Str	11970 SVDP	1330 Reg	4		
26	74	3.0	19500	1/3	28	1.8	0	22	30	84	84	0	0	0	12	0	0	0	2	0	0	0	0	0	Str	16380 SVDP	3650 Reg	5		
27	80	3.8	18500	4/7	24	1.5	0	22	15	35	90	0	0	0	5	0	0	0	5	0	0	0	0	0	Str	16650 SVDP	6475 Reg	6		
28	80	3.8	19700	1/6	14	7/8	0	15	20	10	75	0	0	0	16	0	0	0	18	0	0	0	0	0	Str	14775 MDPP	7880 Reg	7		
29	80	3.8	19700	1/4	22	1/4	0	21	27	37	85	0	0	0	7	0	0	0	10	0	0	0	0	0	Str	13600 SVDP	5920 Reg	8		
30	80	3.8	19700	1/4	22	1/4	0	21	27	37	85	0	0	0	5	0	0	0	10	0	0	0	0	0	Str	13600 SVDP	5920 Reg	9		
31	80	3.8	19700	1/4	22	1/4	0	21	27	37	85	0	0	0	5	0	0	0	10	0	0	0	0	0	Str	13600 SVDP	5920 Reg	10		
32	80	3.8	19700	1/4	22	1/4	0	21	27	37	85	0	0	0	5	0	0	0	10	0	0	0	0	0	Str	13600 SVDP	5920 Reg	11		
33	80	3.8	19700	1/4	22	1/4	0	21	27	37	85	0	0	0	5	0	0	0	10	0	0	0	0	0	Str	13600 SVDP	5920 Reg	12		
34	80	3.8	19700	1/4	22	1/4	0	21	27	37	85	0	0	0	5	0	0	0	10	0	0	0	0	0	Str	13600 SVDP	5920 Reg	13		
35	80	3.8	19700	1/4	22	1/4	0	21	27	37	85	0	0	0	5	0	0	0	10	0	0	0	0	0	Str	13600 SVDP	5920 Reg	14		
36	80	3.8	19700	1/4	22	1/4	0	21	27	37	85	0	0	0	5	0	0	0	10	0	0	0	0	0	Str	13600 SVDP	5920 Reg	15		

Fig. 5.—Shiftogram of abscesses of the liver, pyemia. All hemograms illustrate Type 2.

*Hemogramic Diagnosis:* Acute suppurative inflammation, probably abscess formation (clinically: sepsis of unknown origin).

*Hemogramic Prognosis:* Doubtful, dependent on the location of the suppuration and its treatment.

Treatment suggested: Establishment of free drainage.

This condition was called acute because of the high leukocytosis, neutrophilia and the regenerative left shift. The relatively high percentage of lymphocytes (20 per cent), associated with a high leukocytosis and a wide left shift indicated an infectious process of some duration and suggested an attempt at healing.



The course of the disease traced by the shiftograph, pointed to a complicating septicemia in Hemogram 4. Nonspecific immunotransfusion was recommended and given. This forced the shift strongly to the right within twenty-four hours. It then fluctuated slightly for the next few days, due probably to extension of the infection. Following laparotomy the shiftograph extended widely to the left, terminating in a severe leukemoid left shift with the death of the patient.

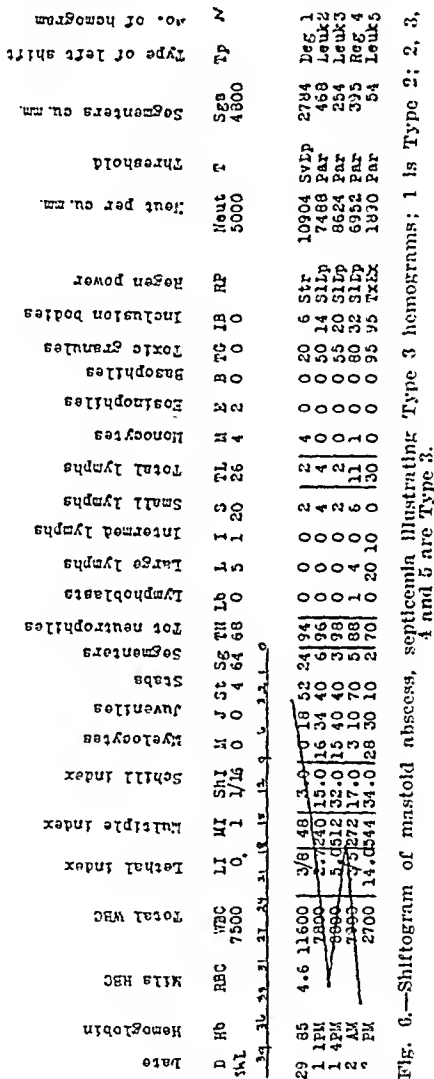


Fig. 6.—Shiftogram of mastoid abscess, septicemia illustrating Type 3 hemograms; 1 is Type 2; 2, 3, 4 and 5 are Type 3.

**Type 3.**—Acute mastoiditis in a white female aged twenty-one years. The first hemogram was made eighteen hours after the patient developed clinical signs suggestive of acute mastoiditis. A bilateral mastoidectomy was done the same day. After a short postoperative improvement acute septicemia developed and the patient died within two days.

**Hemography:** Although the first hemogram is of the second type, the next four show this to be a case of lethal acute irritation, a Type 3. The first count, taken just prior to operation, was interpreted as follows:

**Hemogramic Diagnosis:** Acute suppurative inflammation (clinically: mastoiditis).

**Hemogramic Prognosis:** Hopeful but depends on immediate drainage of the primary focus of infection.

Treatment suggested: Prompt establishment of free drainage.

The first hemogram showed a severe acute irritation by the presence of leukocytosis, high relative neutrophilia, extreme lymphopenia and the wide qualitative and quantitative left shift of the regenerative type. The prognosis was more grave because of the disproportion between the slight leukocytosis and the wide left shift.

The second hemogram was taken just following operation, and showed a transition to Type 3, the so-called lethal irritation, with a falling total white count, no higher than normal,

Date	Hemoglobin	Mls RBC	Total WBC	Lethal Index	Multiple Index	Schill Index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophils	Lymphoblasts	Large lymphs	Intermed lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Regen power	Neut per cu.mm.	Threshold	Sgs td	Type of left shift	No. of hemogram	
6	80	4.3	6900	2/3	80	5/3	10	20	42	14	186	0	12	2	0	14	0	0	0	90	25	SVDP	5160	SVDP	840	Leuk	1	
7	7300	7300	4.3	7300	1/5	55	3/5	1	5	75	5	186	0	10	2	2	14	0	0	0	80	40	SVDP	6278	SVDP	368	Reg	2
8	10900	10900	4.3	10900	1/4	56	3/5	0	10	60	20	90	0	2	2	4	8	0	0	0	40	12	Str	9810	SVDP	2180	Reg	3
9	9900	9900	4.3	9900	1/4	48	3/5	0	10	54	22	88	2	4	2	4	12	0	0	0	20	0	Str	8712	SVDP	2178	Reg	4
10	10000	10000	4.3	10000	1/4	50	3/5	0	10	62	20	82	2	4	0	8	14	4	0	0	0	0	Str	8200	SVDP	2000	Reg	5
11	80	4.0	10900	1/5	16	1/5	0	2	30	32	64	4	16	0	12	32	2	2	0	0	0	0	Str	6976	SVDP	3488	Reg	6
12	9800	9800	4.0	9800	0	7	2/5	0	0	22	63	78	0	16	0	0	16	2	4	0	0	0	Str	7644	Good	5488	Reg	7
13	11800	11800	4.1	11800	1/1	16	1/5	0	6	25	53	64	0	18	2	16	36	0	0	0	0	0	Str	7360	SVDP	3795	Reg	8
14	12000	12000	4.1	12000	1/1	11	2/3	2	6	19	38	62	0	6	2	24	32	6	0	0	0	0	Str	7440	SVDP	4356	Reg	9
15	80	4.1	8000	0	3	1/5	0	0	11	55	166	0	10	0	20	30	1	0	0	0	0	0	Str	5280	Good	4400	Reg	10

Fig. 7.—Shifftogram of lobar pneumonia, delayed resolution, illustrating Type 2, 3, 4, and 5 are Type 2; 6, 7, 8, 9, and 10 are Type 4; 1 and 2 are Type 3.

Fig. 7.—Shiftogram of lobar pneumonia, delayed resolution, illustrating Type 3 hemograms; 1 and 2 are Type 3; 3, 4, and 5 are Type 2; and 6, 7, 8, 9, and 10 are Type 4.

accompanied by a left shift becoming more severe. The interpretation of the second hemogram was as follows:

*Hemogramic Diagnosis:* Septicemia.

*Hemogramic Prognosis:* Fatal in twenty-four hours if the septicemia is not combated.

Treatment suggested: Nonspecific immunotransfusion.

The diagnosis of septicemia was justified by the falling white count, leukemoid left shift, and lethal index of over one. Following a Type 2 hemogram, we interpreted it as toxic exhaustion and bone marrow collapse with a fatal prognosis.

The treatment suggested could not be adopted and the patient died within the predicted twenty-four hours.

*Type 3.*—Bilateral lobar pneumonia in a white male, aged eighteen years. The first hemogram was taken on the day of admission, the sixth day of illness. On this day a pseudocrisis occurred; the temperature dropped to normal, but the pulse remained high and the patient looked very badly. On the seventh day, the patient's condition being even less hopeful, several small nonspecific immunotransfusions were given within the succeeding seventy-two hours. Recovery was uneventful.

*Hemography:* This shiftogram shows the first two hemograms to be a Type 3 lethal picture of toxic exhaustion, with a transition to three hemograms of the Type 2 neutrophilic phase of defense, and terminating with five Type 4 of healing and convalescence. The hemogramic diagnosis, prognosis and suggestion for treatment were based on the first hemogram.

*Hemogramic Diagnosis:* Acute infection, septicemia, no defense (clinically: lobar pneumonia).

*Hemogramic Prognosis:* If postcrisis, fatal in forty-eight hours, unless immediate strenuous treatment is instituted.

Treatment suggested: Nonspecific immunotransfusions.

The condition was called acute infection because of the neutrophilia, lymphopenia, wide qualitative and quantitative left shift and high Schilling and multiple indices. Septicemia was suggested by the high percentage of toxic granules and inclusion bodies, with myelocytes almost equal to segmenters, giving a lethal index of  $\frac{2}{5}$ . No defense, or very low resistance, and toxic depression of the bone marrow was shown by the low total white count of 6,000, neutrophiles 5,160, and segmenters only 840, for protection against a pneumococcus septicemia. The conditional prognosis was justified by the severe depression of the threshold and regenerative power of the bone marrow, giving the defensive force a very poor quality. The Schilling index of 5, the multiple index of 80, the lethal index approaching one and nearly 100 per cent toxic granules pointed to an early termination, unless the toxic pressure was rapidly reduced. Nonspecific immunotransfusion was suggested as in our experience it had been the most effective treatment for septicemia.

The second hemogram is Type 3, but of less severity. It was taken after several small transfusions had been given. The quantitative shift had increased but the more important qualitative shift had turned sharply to the right. Myelocytes and juveniles had decreased markedly in number, while the stabs which are nearly as efficient as segmenters, had increased. While the shift to the right had occurred, without the usual increase in the number of segmenters, it was a qualitative right shift, nevertheless, and denoted improvement. The lethal index had decreased from  $\frac{2}{5}$  to  $\frac{1}{5}$ .

Hemograms 3, 4, and 5 still showed the condition of the patient to be serious but improved. The neutrophilic phase of defense continued active with the neutrophilia and lymphopenia. The regenerative power was stronger with neutrophiles up to 8,000 per c.mm. The multiple index had dropped from 256 to 50. The myelocytes had practically disappeared, but the juveniles were still high. Hemogram 6 changed abruptly from Type 2 to Type 4, from the active neutrophilic phase of defense to the lymphatic immunohealing phase of convalescence. In percentage the neutrophiles were about normal, but a relative and absolute lymphocytosis with a lymphatic left shift occurred. Toxicity had been reduced or eliminated. The regenerative power and threshold were improved. The neutrophiles remained around 7,000 and the segmenters had risen to 4,000. All indices had been reduced, and the toxic granules and inclusion bodies had disappeared. Except for a temporary setback, the shiftograph showed a progressive right shift to convalescence and recovery of which Hemogram 10 is a typical example.

*Type 4.*—White male, aged forty-five years, with a history of lassitude, dullness, lack of energy, anorexia, and a slight loss of weight. He occasionally ran a slight afternoon elevation of temperature. The condition cleared up one week after an abscessed tooth had been extracted.

*Hemography:* Both hemograms of this shiftogram are Type 4, showing a high normal white count, neutropenia, lymphocytosis, monocytosis, and slight left shift.

*Hemogramic Diagnosis:* Subacute infection (teeth, tonsils or pharynx). (Clinically: possible focus of infection.)

*Hemogramic Prognosis:* Good.

Treatment suggested: Eliminate any foci of infection.

Date	Hemoglobin	Miles RBC	Total WBC	Lethal Index	Multiple Index	Schill Index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophils	Lymphoblasts	Large lymphs	Intermed lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Regen power	Neut per cu mm	Threshold	Segmenters cu mm	Type of left shift	No of hemogram
14 100	95	5.6	8900	0 4.0	0 4.0	1 1/2	0 2	8 36	148	0 16	0 16	0 16	0 16	0 16	0 16	0 16	0 16	0 16	0 16	0 16	0 16	0 16	0 16	0 16	0 16	0 16	0 16
21 95	9500	4.8	9500	0 2.6	0 2.6	1 1/2	0 0	6 36	142	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8

Fig. 8.—Shiftogram of abscessed tooth, illustrating two Type 4 hemograms.

Date	Hemoglobin	Miles RBC	Total WBC	Lethal Index	Multiple Index	Schill Index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophils	Lymphoblasts	Large lymphs	Intermed lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Regen power	Neut per cu mm	Threshold	Segmenters cu mm	Type of left shift	No of hemogram
9 80	4.0	5400	0 32	2.0	0 32	2.0	0 0	40 17	64	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
11 10400	16	1.0	0 16	1.0	0 16	1.0	0 0	34 32	66	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4
21 13700	0 9	4/7	0 9	4/7	0 9	4/7	0 0	28 48	76	0 6	0 6	0 6	0 6	0 6	0 6	0 6	0 6	0 6	0 6	0 6	0 6	0 6	0 6	0 6	0 6	0 6	0 6

Fig. 9.—Shiftogram of gumma of the liver illustrating Type 5-A hemogram: 1 is Type 5-A, 2 and 3 are Type 4.

The first hemogram was made before the patient's focus of infection had been suspected. The diagnosis of subacute infection was made because of the slight left shift of 4 multiples, bordering on the regenerative type, as it included two juveniles. The monocytosis and lymphocytosis, with a left shift of lymphocytes to 16 of the large type, also pointed to the fairly acute lymphatic type of irritation which is seen in low grade infections.

The second hemogram was made one week after the abscessed tooth had been extracted. The total white count was about the same, but the neutrophils had shifted to the right to shift had occurred. The number of small lymphocytes had doubled, indicating the lymphatic healing phase.

*Type 5-A.*—Gumma of the liver in a white female, aged thirty-nine years with pain in the right upper quadrant for several years. The patient's symptoms were gradually becoming worse; the liver was enlarged and tender. Blood Wassermann was positive. Operation disclosed the gumma of the liver, confirmed by biopsy. The patient improved with antisyphilitic therapy.

*Hemography:* The first hemogram of this shiftogram, on which the diagnosis was based, was a Type 5-A.

*Hemogramic Diagnosis:* Chronic suppurative hyperplastic inflammation (probably syphilis or tuberculosis). (Clinically: gumma.)

*Hemogramic Prognosis:* Poor.

Active inflammation was shown by the presence of more than 35 shift cells, a fairly wide degenerative shift with only 918 segmenters per c.mm. out of a total of 3,078 neutrophils. Chronicity was shown by the lymphocytosis of 37 per cent, all of the small type. This was a lymphatic right shift and indicated efforts at healing. Hemograms 2 and 3 are Type 4. They followed the operation which disclosed gumma of the liver and showed the reaction of the patient to surgery; and the subsequent attempt at immunohealing.

Date	Hemoglobin	Mils RBC	Total WBC	Lethal index	Multiple index	Schill index	Myelocytes	Juveniles	Stabs	Segmentors	Tot neutrophils	Lymphoblasts	Large lymphs	Intermediate lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Regen power	Heat per cu.mm.	Threshold	Segmentors cu.mm	Type of left shift	No of hemogram
D	Hb	RBC	WBC 7500	LI 0	MI 1	Sh I 1/16	M 0	J 0	St 4	Seg 6	TN 68	Lb 0	L 5	I 1	S 20	TL 26	M 4	E 2	B 0	TC 0	IB 0	RP	Heat 5000	T 7	Segs 4800	TP	N
Sh I	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
7	80	4.0	7200	1/12	12	4/5	4	6	30	50	30	0	2	0	4	6	0	4	0	10	2	Str	6480	M4Ep	3503	Reg	1
8			5500	0	19	1.2	0	0	2	20	44	0	52	0	0	52	0	0	0	50	14	SvEp	2420	SvEp	1100	Le	2
9			5000	1/12	56	3.5	0	0	2	12	54	0	16	0	24	40	4	0	0	0	6	SvEp	2700	SvEp	600	Reg	3
10			5100	0	8	1/2	0	0	18	34	52	0	26	0	22	48	0	0	2	50	0	SvEp	2652	SvEp	1734	Le	4
11			5000	0	8	1/2	0	0	24	26	70	0	0	0	18	24	0	0	4	0	0	SvEp	3500	SvEp	2300	De	5
12			4800	0	20	1.3	0	0	34	26	60	0	0	0	32	40	0	0	0	0	0	SvEp	2880	SvEp	1248	De	6
13			4500	0	17	1.1	0	0	30	26	63	0	0	0	30	30	0	0	0	0	0	SvEp	2835	SvEp	1350	De	7
14	85	4.3	4500	0	18	1/2	0	0	24	51	75	0	0	0	28	20	0	0	0	0	0	M4Ep	4125	M4Ep	2320	De	8
15			5500	0	8	1/2	0	0	24	51	75	0	0	0	28	20	0	0	0	0	0	M4Ep	4125	M4Ep	2320	De	9

Fig. 10.—Shiftogram of influenza illustrating Type 5-B hemogram; 2, 3, 4, 6, and 7 are Type 5-B, 1 is a Type 2, and 5 and 8 are Type 4.

*Type 5-B.*—Influenza in a white male, aged twenty-eight years. The patient ran a typical course with headache, malaise, chills and fever, and cough. On January 10 patient was discharged apparently well but readmitted the following day with a return of symptoms. He was finally discharged in good health on January 15.

*Hemography:* All hemograms except the first, fifth and eighth on this shiftogram are Type 5-B, with a low normal white count, neutropenia lymphocytosis and a fairly wide left shift. The first hemogram is a Type 2, showing a high neutrophilia.

*Hemogramic Diagnosis:* Acute infection (clinically: influenza).

*Hemogramic Prognosis:* Good.

Acute infection was diagnosed from the first hemogram which showed the high neutrophilia and extreme lymphopenia common to all infections. Influenza was indicated by the second hemogram, which presented the low white count, neutropenia, lymphocytosis and lymphatic left shift characteristic of this disease when considered with the clinical symptoms.

In tracing the course of the disease the shiftograph showed a left shift peak on the third day, seen quite frequently in this condition, followed by a right shift apparently of a convalescent nature on the fifth day. A wide left shift on the sixth day, coinciding with the clinical relapse, was followed by a continuous right shift to a beginning convalescence on the ninth day.

DIFFERENTIAL DIAGNOSIS

The noninfectious, infectious or toxic nature of many conditions may be difficult to distinguish clinically. In these cases hemography may be of great

Diagnosis	Hb	Mils RBC	Total WBC	Lethal index	Multiple index	Schill index	Myelocytes	Juveniles	Stabs	Segmenters	Total neutrophils	Lymphoblasts	Large lymphs	Intermed lymphs	Small lymphs	Total lymphs	Monocytes	Eosinophiles	Basophiles	Toxic granules	Inclusion bodies	Regen power	Neut per cu. mm.	Threshold	Segmenters cu. mm.	Type of left shift	
			7500	LI	MI	SHI	M	J	St	Sg	TH	Lb	L	I	S	TL	M	E	B	TC	TP	RP	Neut	†	Sg	TP	
A. Incarcerated hernia	80	4.0	20800	0	2.6	1/6	0	0	14	63	197	0	3	0	0	3	0	0	0	0	0	0	Str	20176	SLDp	17264	Des
B. Strangulated hernia	80	4.2	24500	1/923.		1.8	4	4	56	34	96	2	0	0	0	2	0	0	0	100	40	Str	24020	SvDp	8330	Reg	
A. Ovarian cyst twisted on pedicle			19600	0	10.	2/3	0	0	20	58	76	0	2	0	14	16	6	0	0	0	0	0	Str	15288	SLDp	11368	Des
B. Rup. appendicial abscess			17600	0	48.	3.0	0	2	66	22	90	0	4	0	4	8	2	0	0	25	6	Str	15840	SvDp	3872	Reg	
A. Tumor of lung			12000	0	4.	1/4	0	0	16	64	80	0	12	0	2	14	2	4	0	0	0	0	Str	9600	SLDp	7680	Des
B. Costo-phrenic abscess			30700	0	40.	2.5	0	0	54	22	78	0	6	0	0	6	14	0	2	0	0	0	Str	23946	SvDp	6754	Des

FIG. 11.—Hemograms showing differential diagnosis of (A) noninfectious and (B) infectious processes.

assistance to the clinician for infectious processes with similar clinical pictures, falling into the same type of hemogram, are difficult to differentiate hemograph-

ically, while those falling into different hemogramic types are easily differentiated. The following three comparisons are used for illustration:

(A) is a Type 1, and (B) a Type 2 hemogram in each case. They both show a high leukocytosis, high neutrophilia, and marked lymphopenia.

Diagnosis	Type of left shift										Type of left shift.													
	Total WBC	Lethal index	Multiple index	Schill index	Myelocytes	Juveniles	Stabs	Segmenters	Not neutrophils	Lymphoblasts	Large lymphs	Informed lymphs	Small lymphs	Total lymphs	Monocytes	Koanophiles	Basophilics	Toxic granules	Inclusion bodies	Hegen power	Hour per cu. mm	Threshold	Segs	dt
Influenza	5500	0.19	1.2	0	0	24	20	44	0	52	0	4	0	50	14	SvDp	2420	SvDp	1100	DeG				
Lobar pneumonia	23100	1/14	35	2.2	2	8	54	28	92	0	3	0	0	0	0	0	0	0	0	Str	21452	SvDp	6468	DeG

Fig. 12.—Hemograms showing differential diagnosis of processes that may be similar clinically but of different hemogramic types.

Diagnosis	Type of left shift.										Type of left shift.														
	Total WBC	Lethal index	Multiple index	Schill index	Myelocytes	Juveniles	Stabs	Segmenters	Not neutrophils	Lymphoblasts	Large lymphs	Intermed lymphs	Small lymphs	Total lymphs	Monocytes	Koanophiles	Basophilics	Toxic granules	Inclusion bodies	Hegen power	Neutrophils cu. mm.	Threshold	Segs	dt	
Suppurative salpingitis	19000	0	13	6/7	0	0	38	42	60	0	8	0	10	10	2	0	0	0	0	0	Str	15200	Madp	7980	DeG
Supp. gangrenous appendicitis	17850	0	10	2/3	0	2	36	54	62	0	2	0	6	6	0	0	0	0	0	0	Str	16376	Madp	9612	DeG

Fig. 12.—Hemograms showing differential diagnosis of processes that may be similar clinically but of different hemogramic types.

Fig. 13.—Hemograms showing differential diagnosis of processes that are similar clinically and of the same hemogramic types.

(A), however, shows but slight depression of the bone marrow threshold. The slight left shift is degenerative, the Schilling index a fraction; the multiple index only a few times greater than normal and the large army of neutrophiles consists almost entirely of efficient phagocytes or segmenters.

(B), on the contrary, shows many indications of toxic depression of the threshold and lowered resistance, consisting of a marked left shift, Schilling index greater than one and a multiple index many times greater than normal. The segmenters number less than half the total neutrophils, so that while the cellular defensive force is large, it is of very poor quality.

The first hemogram is Type 5-B; the second is Type 2. Both were taken at the onset of disease before diagnostic clinical signs had developed. Depression of the threshold is not a dependable means of distinguishing between these two infections. Although the character of the shift differs in the two cases cited, it cannot be used as a differential point because the quality and quantity of the shift is often the same in the two diseases. The different action of the toxins on the reproductive power of the bone marrow is shown in the two hemograms. In influenza severe depression or lack of stimulation of the regenerative power results in a severe neutropenia with 44 per cent neutrophils, or 2,420 per c.mm. In pneumonia strong stimulation of the regenerative power is shown by the 92 per cent, or 21,452 neutrophils per c.mm., a marked neutrophilia. Thus a wide left shift, leukopenia, neutropenia, and lymphocytosis suggest influenza, while leukocytosis, neutrophilia, and lymphopenia, with a wide left shift, indicates pneumonia.

These hemograms are both Type 2, and are similar in practically all respects, except the degree of neutrophilia and lymphopenia. The hemogramic diagnosis in this case is difficult and often impossible. However, the total white count with 80 per cent neutrophils, and 20 per cent lymphocytes and only a moderate degenerative left shift, is more characteristic of acute salpingitis than of appendicitis. If the first count had been due to acute suppurative appendicitis, the toxic depression of the threshold accompanying a total white count of 19,000 would have been greater. Thus, there would have been a wider shift, at least of the regenerative type, with a neutrophilia of probably 90 per cent and a corresponding lymphopenia. The second hemogram illustrates the type of shift that accompanies a high leukocytosis in acute appendicitis and shows the difference between the two.

#### COMMENT

We have endeavored to establish the following points:

1. The Schilling system of differentiating white blood cells should supercede all other forms of differential count. It reflects more consistently and accurately the degree of intoxication or infection of the patient.

2. Our modification of the Schilling hemogram has made possible an easier and more accurate analysis of the hemogram;

- a. By adding a shiftograph to trace at a glance the extent of the quantitative shift indicated by the Schilling index.

- b. By adding a multiple index to show the number of times a given shift is greater than normal.

- c. By adding a lethal index to show the quality of the shift in a ratio of myelocytes or juveniles to segmenters, and to aid in prognosis. A fatal outcome almost always results when the lethal index approaches one and the multiple index 100.



d. By using the symbol "RP," to record the degree of stimulation, depression, or paralysis of the bone marrow, reflected by the number of neutrophiles per cubic millimeter.

e. By listing the neutrophiles per cubic millimeter to show the numerical strength of the neutrophilic army of defense.

f. By using the symbol "T" to record the condition of the bone marrow threshold, estimated by the quality of the shift. The degree of depression of the threshold when compared with the ratio of segmenters to neutrophiles per cubic millimeter, indicates the quality and quantity of the cellular army of defense.

g. By listing the segmenters per c.mm. for comparison, with average normals, and with neutrophiles per c.mm.

h. By routinely Schillingizing the lymphocytes a vast field of new information is opened up. Diseases of a chronic character, having a tendency to lymphocytosis, in the active phase show a lymphatic left shift with a predominance of large lymphocytes, and in the latent or healing phases a right shift to small lymphocytes.

In acute infections the first sign of the immunohealing phase of convalescence is an increase in lymphocytes and a lymphatic left shift with a predominance of large lymphocytes. As convalescence progresses the lymphocytes shift to the right with small lymphocytes predominating.

In tuberculosis the variation in total lymphocytes, the lymphatic shift in conjunction with the neutrophilic shift, and the rise and fall of monocytes and eosinophiles, definitely assist in classifying the various phases of the disease and in detecting complications.

i. By showing that the Schilling index alone should be used with caution as a diagnostic and prognostic aid. It represents the nonfilament-filament ratio, and is valuable only when considered with the quality of the shift.

The total number of shift cells alone may be used in differentially diagnosing acute appendicitis. In general with a neutrophilia present, and a Schilling index of one or over, shift cells 15 to 35 indicate acute catarrhal appendicitis; 35 to 50 suppurative appendicitis; 50 to 60 abscess in appendix; and over 60 ruptured appendix with general peritonitis.

j. By listing Doehle's inclusion bodies and Freyfeld's toxic granules in percentage of neutrophiles of all types containing them. These are found in any severe acute infection or intoxication and assist in determining the presence of septicemia.

3. Disease entities and different types of the same disease may be classified according to the type of hemograms which they show, thus facilitating diagnosis and prognosis.

4. Need for the shiftogram, a chart of sequential hemograms joined by the shiftograph, in following the daily progress of a disease. Definite changes in the course of an infection are usually indicated by a turn of the shiftograph often before there is any clinical change. During the course of a chronic infection an increase in neutrophiles with neutrophilic and lymphatic left shift indicates an acute exacerbation. A change in the severity of the picture during an acute infection may indicate a complication. Similarly a sudden turn of

the shiftograph and qualitative shift to the left, during a convalescent picture, may indicate a relapse. Remissions and attacks, in diseases characterized by them, may be similarly predicted by the change in the hemogram.

Lobar pneumonia seems to be an outstanding exception to the use of the shiftograph in predicting the turning point in the course of an infection. At the time of the clinical crisis the hemogram may show its greatest severity, the right shift not appearing until twenty-four to forty-eight hours later.

A shiftogram showing a sustained leukocytosis of from 10,000 to 20,000 with a neutrophilia of 8,000 to 18,000 per c.mm., a degenerative or regenerative left shift and Schilling index over one, is usually indicative of confined pus, e.g., abscess, empyema, etc.

A shiftogram has a place in the control of treatment by specific and non-specific immunotransfusions, foreign protein therapy, diathermy, vaccines, anti-toxins, etc. Many of these methods, which produce a febrile reaction clinically, also cause fairly characteristic changes in the blood picture and all of them may be more accurately controlled by the shiftogram.

A consideration of the clinical facts is important before an accurate hemogramic diagnosis and prognosis may be made. The clinical findings are important for prognosis in general because the hemogram has a different interpretation depending on the location of the infection. The same severity of blood picture that might indicate a fatal prognosis in intracranial infections, grave in pulmonary, serious but hopeful in abdominal, would cause but slight concern in pelvic conditions. Thus a slight shift in intracranial infections becomes of relatively great importance. A better prognosis may be given if it is known that adequate medical or surgical treatment is possible to control the primary focus of infection.

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## A NOTE ON THE USE OF THE HAGEDORN-JENSEN BLOOD SUGAR TECHNIC IN CASES OF PHLORHIZINIZATION\*

S. B. BARKER, B.S., NEW HAVEN, CONN.

**D**ESPITE the generally accepted fact that the injection of phlorhizin causes a fall in blood sugar, examination of the literature reveals that comparatively high values have been reported, by Coolen,<sup>1</sup> Pavy,<sup>2</sup> Biedl and Kolisch,<sup>3</sup> Massaut,<sup>4</sup> and Houssay and Biasotti.<sup>5</sup> Yamada<sup>6</sup> seems to have been the first definitely to associate high values with reducing action of phlorhizin itself rather than with any glycemie action of the drug. He found an apparent rise of blood sugar as determined by the Hagedorn-Jensen ferrieyanide method, but no change for several hours with Bang's copper reduction technic. Lundsgaard<sup>7</sup> precipitated the blood proteins with mercuric sulphide, and found that phlorhizin was removed by this procedure.

### METHODS

Total reducing substances were determined by the Hagedorn-Jensen technic,<sup>8</sup> following a Somogyi zinc precipitation.<sup>9</sup> Nonfermentable reducing substances were determined on the zinc filtrate after fermentation with yeast according to Peters and Van Slyke.<sup>10</sup>

### EXPERIMENTAL

The error caused in the Hagedorn-Jensen method is twofold: the phlorhizin reduces ferrieyanide and raises the titration value by combining with iodine. The present work is an attempt to study and rectify both of these errors.

To determine the iodine absorption, a 0.1 per cent water suspension of phlorhizin (Merek) was used in place of blood. After the Somogyi precipitation, 1 c.c. samples were placed with 1 c.c. portions of an iodine solution for five, ten,

\*From the Laboratory of Physiology, Yale University. The School of Medicine.  
Received for publication, January 24, 1934.

and fifteen minutes, both at 25° C. and at 18° C. The titration values with 0.005 N  $\text{Na}_2\text{S}_2\text{O}_3$ , as used in the Hagedorn-Jensen technic, are given in Table I.

TABLE I  
IODINE ABSORPTION PER C.O. OF 0.1 PER CENT PHLORHIZIN FILTRATE

	IODINE ABSORBED AT 25° C. (AS C.G. 0.005 N $\text{Na}_2\text{S}_2\text{O}_3$ )	IODINE ABSORBED AT 18° C. (AS C.G. 0.005 N $\text{Na}_2\text{S}_2\text{O}_3$ )
After 5 minutes	0.10	0.02
After 10 minutes	0.22	0.02
After 15 minutes	0.24	0.04

Examination of Table I shows that good cooling and speedy titration will minimize the error due to absorption of iodine by phlorhizin.

Using the information thus obtained, 1 c.c. samples of the filtrate were then subjected to the entire technic for the detection of reducing substances, both before and after fermentation. The values obtained are presented in Table II.

TABLE II  
REDUCING POWER PER C.C. OF 0.1 PER CENT PHLORHIZIN SUSPENSION

	REDUCING POWER (AS MG. GLUCOSE)
Total	0.39
After 10 minutes' fermentation	0.36
After 15 minutes' fermentation	0.37
After 20 minutes' fermentation	0.36

Phlorhizin, therefore, has a constant reducing power even after as much as twenty minutes' fermentation. The disastrous effects of over fermentation are shown in Table III, which contains reducing values of blood filtrates after one-half hour with yeast. The data show that fermentation of the phlorhizin must have taken place.

TABLE III  
REDUCING SUBSTANCES IN BLOOD WITH PHLORHIZIN, AFTER THIRTY MINUTES' FERMENTATION  
(AS MG. GLUCOSE PER C.C.)

PHLORHIZ. MG./C.C.	TOTAL REDUCING CONTROL	TOTAL REDUCING EXPER.	FERMEN. REDUCING CONTROL	FERMEN. REDUCING EXPER.
1	0.77	1.12	0.73	0.85
2	0.77	1.46	0.73	0.92
3	0.77	1.81	0.73	1.00
4	0.77	2.19	0.73	1.12

Putting to use the facts shown by these experiments, namely, that phlorhizin is not fermented in fifteen minutes, and that at a lowered temperature it absorbs very little iodine, the effect of phlorhizin on whole blood was next determined. For this, oxalated slaughter-house blood, to which known amounts of phlorhizin had been added, was used, total and nonfermentable reducing substances being determined. Table IV contains the values obtained.

The dose of phlorhizin usually administered to animals is 1 gram per 10 kilos of body weight. If this entire amount passed at once into the blood stream, the maximum concentration there would be about 1.3 mg. per c.c. If the entire dose became distributed throughout all the tissues, the minimal concentration

TABLE IV  
REDUCING SUBSTANCES IN BLOOD WITH VARYING AMOUNTS OF PHLORHIZIN  
(AS MG. GLUCOSE PER C.C.)

PHLORHIZIN MG./C.C. (1)	TOTAL REDUCING SUB- STANCES CONTROL (2)	TOTAL REDUCING EXPER. (3)	DIF- FERENCE (3)-(2)	NON- FERMEN. REDUCING CONTROL	NON- FERMEN. REDUCING EXPER.	FERMEN. REDUCING CONTROL (4)	FERMEN. REDUCING EXPER. (5)	DIF- FERENCE (5)-(4)
0.10	1.13	1.15	0.02	0.10	0.14	1.03	1.01	-0.02
0.10	1.24	1.24	0.00	0.11	0.11	1.13	1.13	0.00
0.13	1.13	1.15	0.02	0.10	0.12	1.03	1.03	0.00
0.13	1.24	1.29	0.05	0.11	0.17	1.13	1.12	-0.01
0.17	1.13	1.17	0.04	0.10	0.14	1.03	1.03	0.00
0.17	1.29	1.34	0.05	0.11	0.18	1.18	1.16	-0.02
0.20	1.13	1.19	0.06	0.10	0.14	1.03	1.05	+0.02
0.25	1.13	1.19	0.06	0.10	0.16	1.03	1.03	0.00
0.25	1.29	1.35	0.06	0.11	0.16	1.18	1.19	+0.01
0.33	1.24	1.35	0.11	0.11	0.14	1.13	1.11	-0.02
0.33	1.24	1.36	0.12	0.11	0.24	1.13	1.12	-0.01
0.50	1.08	1.27	0.19	0.14	0.30	0.94	0.97	-0.03
0.50	1.29	1.45	0.16	0.11	0.26	1.18	1.19	+0.01
1.00	1.08	1.41	0.33	0.14	0.48	0.94	0.93	-0.01
1.00	0.48	0.77	0.29	0.05	0.32	0.43	0.45	+0.02
1.00	0.48	0.79	0.31	0.05	0.36	0.43	0.43	0.00
1.50	1.29	1.72	0.43	0.11	0.51	1.18	1.21	+0.03
1.50	1.29	1.77	0.48	0.11	0.55	1.18	1.22	+0.04
2.0	1.75	2.36	0.61	0.14	0.67	1.61	1.69	+0.08
2.0	1.08	1.72	0.64	0.14	0.71	0.94	1.01	+0.07
4.0	1.75	2.96	1.21	0.14	1.21	1.61	1.75	+0.14
6.0	1.75	3.38	1.63	0.14	1.81	1.61	1.85	+0.24

of phlorhizin in the blood would be about 0.1 mg. per c.c. The amounts of phlorhizin added to the blood were calculated to cover the range one would expect to encounter after phlorhizinization.

It has been shown that the presence of phlorhizin in blood causes an apparent increase in sugar content, under the usual conditions for the Hagedorn-Jensen determination. This fact casts considerable doubt upon the accuracy of blood sugar values determined by the Hagedorn-Jensen method during phlorhizin injections.

#### SUMMARY

1. Phlorhizin introduces two errors into blood sugar determination with the Hagedorn-Jensen technique: first, it reduces ferrieyanide, and, second, it combines with iodine.

2. Fermentation with yeast eliminates the first error, while rapid titration at 18° C. reduces the second.

3. Data are presented to show that the Hagedorn-Jensen method, with the modifications noted, may be used to determine fermentable reducing substances in blood after phlorhizinization.

The author wishes to acknowledge gratefully much valuable advice given by Dr. H. E. Himwich during the course of the work.

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## A STABLE STARCH INDICATOR FOR THE IODOMETRIC ESTIMATION OF CHLORIDES IN BLOOD AND URINE\*

C. S. SHAPIRO, M.A., NEW YORK, N. Y.

THE iodometric method for chlorides in blood is known to possess many advantages over the thiocyanate procedure.<sup>1</sup> It has been found applicable to urines also with high degree of accuracy.<sup>2</sup> It has, however, one objectionable feature, namely, the rapid deterioration of the starch indicator. The condition of the starch has been found to affect the accuracy of this method, since the end-point is sharp only when the starch is freshly made.<sup>3</sup> It is obvious, therefore, that a stable starch indicator would add to the reliability and convenience of this method.

A number of preservatives for starch indicators has been described, but, with the possible exception of salicylic acid,<sup>1</sup> they seem unsuitable for the special case of iodometric estimation of chlorides in biologic fluids.

In our experiments the urine preservative containing acetyl-salicylic acid and hexamethylenetetramine<sup>4</sup> was used as a starch stabilizer with very good results. It did not interfere with the iodometric method for chlorides in any particular. The preserved starch solutions were found to remain stable and efficient as indicators, without any special precautions being necessary. They did not develop any odor and were about as clear as a freshly prepared starch solution. A negligible amount of white precipitate formed in the course of time, but in no way interfered with the end-point. These solutions were kept at room temperature, since in the ice box their contents were found to precipitate out.

The preserved starch indicator was tested in a number of iodometric determinations of chlorides and checked against a freshly prepared starch solution. At the time of the test the preserved starch was from three to five months old, while the fresh starch was renewed every two days. The behavior of the two indicators was compared in standardization of the KI solution, in the analysis of NaCl controls and in the estimation of chlorides in blood and urine specimens. The results obtained show very close agreement and are given in Table I.

In these experiments some of the blood specimens contained NaF and monochlorobenzene as preservative.<sup>5</sup> All urine specimens were preserved with

\*From the Laboratories of the Life Extension Institute.  
Received for publication, January 29, 1934.

The starch used in these experiments was obtained from the Elmer and Amend Co.; the hexamethylenetetramine and acetyl-salicylic acid came from Merck & Co.

200 mg. each of acetyl-salicylic acid and hexamethylenetetramine per 100 c.c. of urine.<sup>4</sup> The last two substances were also added to a part of control NaCl solutions in the same concentration. The urines were all negative for albumin and the preliminary precipitation of the urine proteins was omitted.<sup>2</sup>

#### REAGENTS

In view of possible interaction of the preservative with the buffer salts (sodium citrate and sodium nitrite) on long standing, it was thought safer to prepare the indicator in the form of two solutions (A and B) as recommended by Short and Gellis.<sup>6</sup> To Solution A was added the above preservative. Solution B was left unchanged. The rest of the reagents were the same as used by the above authors and their procedure was followed for both blood and urine.

*Solution A (Preserved Starch).*\*—Bring 100 c.c. of water to boiling. Take off flame and immediately dissolve in it 2.5 gm. of soluble starch with constant stirring. Cool and dilute to 150 c.c. To the 150 c.c. of the cold solution add 510 mg. of acetyl-salicylic acid U.S.P. and 510 mg. of hexamethylenetetramine U.S.P. Stir the mixture thoroughly to insure complete solution. Let stand overnight. By that time some solid material that may be left undissolved will settle to the bottom. Centrifuge for ten minutes at high speed. Decant carefully the supernatant fluid. Keep at room temperature, tightly corked when not in use.

*Solution B (Buffer Salts).*—Dissolve 466 gm. of crystalline sodium citrate and 20 gm. of sodium nitrite in about 800 c.c. of water. Dilute to 1,350 c.c. The two solutions are mixed just before use, in proportion of 1 c.c. of A to 9 c.c. of B.

*Standard Silver Nitrate Solution.*—Dissolve 5.812 gm. of  $\text{AgNO}_3$  in about 400 c.c. of water, using an 800 c.c. beaker. Add exactly 250 c.c. of  $\text{HNO}_3$  (sp. gr. 1.42). Cool to room temperature. Transfer to a liter flask and dilute to mark with water.

*Standard Potassium Iodide Solution M/73.1.*—Dissolve 2.4 gm. of KI in a liter of water. Standardize by titrating against 5 c.c. of the  $\text{AgNO}_3$  solution, to which have been added 5 c.c. of water and 6 c.c. of the starch indicator. Adjust so that the amount required will be 12.65 c.c. (12.50 c.c. to precipitate the silver nitrate and 0.15 c.c. to develop a definite end-point).

#### PROCEDURE

Into a 50 c.c. graduated tube introduce 20 c.c. of Folin-Wu blood filtrate and about 15 c.c. of water (or 1 c.c. of urine and about 35 c.c. of water). Add 10 c.c. of  $\text{AgNO}_3$  solution. Dilute carefully to the mark with water. Mix several times by inversion. Centrifuge for ten minutes at high speed and decant the supernatant fluid through a filter. Titrate 20 c.c. of this filtrate (equivalent to 0.8 c.c. of blood, or to 0.4 c.c. of urine) with the KI solution, using 6 c.c. of the starch indicator. The KI solution is added slowly with constant stirring. Toward the end it is added drop by drop. A white porcelain dish instead of a glass vessel is used for titration. The yellow  $\text{AgI}$  precipitate serves as the correct background for the end-point, which is the first permanent blue.<sup>1</sup> In our

experiments enough of the final filtrate was obtained for duplicate determinations, one of which was made with the preserved starch, the other with the freshly prepared starch.

*Calculation.—*

In the case of blood the formula is:

$$(10.15 - \text{c.c. of KI sol. used}) \times 100 = \text{mg. NaCl per 100 c.c. of blood.}$$

In the case of urine the factor is doubled:

$$(10.15 - \text{c.c. of KI sol. used}) \times 200 = \text{mg. NaCl per 100 c.c. of urine,}$$

since the amount of urine taken is only half that of blood.

TABLE I\*  
COMPARISON OF PRESERVED AND FRESH STARCH INDICATORS

Standardization of KI c.c. KI used in titration	Starch	
	Preserved 12.24	Fresh 12.25
Value required = 12.65 c.c. per 5 c.c. of st. AgNO <sub>3</sub> sol.	12.65 12.67 12.67	12.63 12.65 12.65

CONTROL NaCl SOLUTIONS MG. PER 100 C.C. OF SOLUTION		STARCH PRESERVED FOUND FRESH FOUND		STARCH PRESERVED FRESH % ERROR	
	GIVEN				
	(a) 1,000	998	994	-0.2	-0.6
	(b) 1,000	1,002	998	+0.2	-0.2
Theoretical error (varies with concentration):	(a) 500	494	497	-1.2	-0.6
±1% to ±3%	(b) 500	496	494	-0.8	-1.2
	(a) 250	250	256	0	+2.5
	(b) 250	250	254	0	+1.6

(a) Pure NaCl solutions. (b) NaCl solutions containing acetyl-salicylic acid and hexamethylenetetramine.

BLOOD CHLORIDES MG. PER 100 C.C. OF BLOOD		STARCH PRESERVED FOUND FRESH FOUND		STARCH PRESERVED FRESH % DEVIATION	
The blood was preserved with NaF and Monochlor-benzene		504	501		0.6
		473	477		0.8
		539	535		0.8
Theoretical deviation 1.2-1.5%		514	516		0.4
		405	413		1.8
		387	382		1.3

URINE CHLORIDES MG. PER 100 C.C. OF URINE		STARCH PRESERVED FOUND FRESH FOUND		STARCH PRESERVED FRESH % DEVIATION	
The urine was preserved with acetyl-salicylic acid and hexamethylenetetramine		644	652		1.3
		910	910		0
		546	546		0
		260	266		2.5
Theoretical deviation 1-3%		450	450		0
		700	698		0.3
		558	550		1.6

\*The preserved starch was 3-5 months old during the experiments. It was preserved with acetyl-salicylic acid and hexamethylenetetramine.

NOTE: The fresh starch was renewed every two days.



200 mg. each of acetyl-salicylic acid and hexamethylenetetramine per 100 c.c. of urine.<sup>4</sup> The last two substances were also added to a part of control NaCl solutions in the same concentration. The urines were all negative for albumin and the preliminary precipitation of the urine proteins was omitted.<sup>2</sup>

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## DISCUSSION

Inspection of Table I indicates that acetyl-salicylic acid in combination with hexamethylenetetramine forms a dependable preservative of starch solutions used as indicators. The theoretical error is given in the table. In the estimation of chlorides it varies with concentration of NaCl. It is equal to  $\pm 0.6-1.5$  per cent when 2 c.c. of material is used and is nearly doubled when 1 c.c. is taken for analysis. In our experiments the controls were done in the same way as the urines, 1 c.c. having been used; accordingly, the error here is the same. As to bloods and urines, the deviation between results obtained with the two indicators amounts in our case to the difference between duplicate titrations. It can be seen that the agreement is within theoretical limits.

## SUMMARY

A stable starch indicator was prepared with the use of acetyl-salicylic acid and hexamethylenetetramine as preservative. It was found to keep in the original state for several months. Comparative tests indicate that it can be safely used in iodometric methods for chlorides in blood and urine. The tests further show that this indicator works well when the specimens themselves contain the above mentioned preservatives.

I wish to thank Dr. James J. Short, Director of Laboratories of the Life Extension Institute, New York, N. Y., for having given his help and encouragement in this work.

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# A SIMPLE METHOD FOR THE MANIFOLDING OF KYMOGRAPH TRACINGS<sup>2</sup>

DAVID LUBIN, M.D., BALTIMORE, Md.

IT IS often found desirable to have copies of kymograph tracings for class distribution, and as a means of preserving the original, a method will be described whereby any number of copies may be made photographically without the necessity of using film or plates or camera.

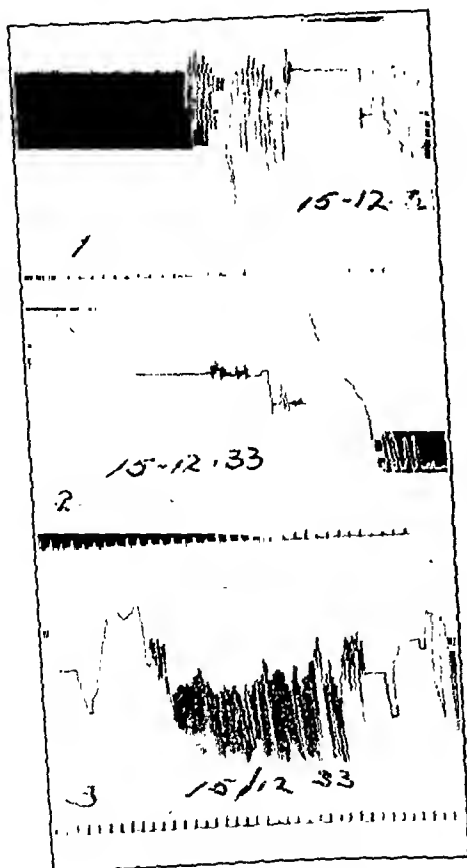


Fig. 1.

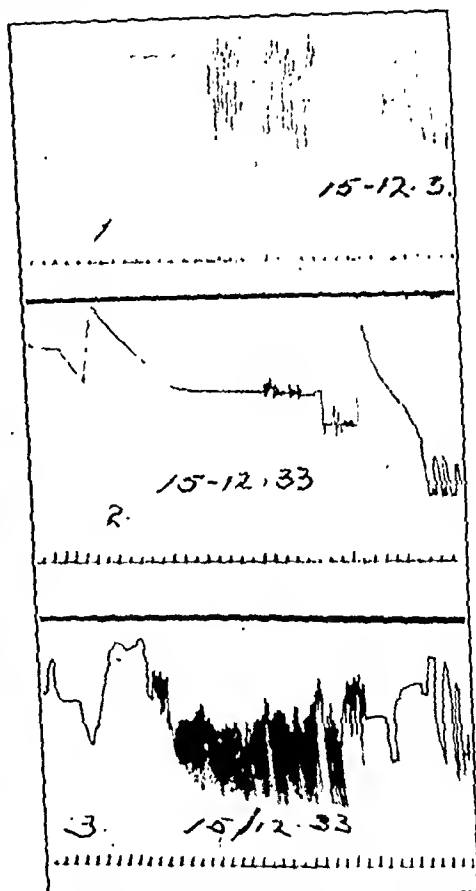


Fig. 2.

Fig. 1.—Drum smoked as described. Tracing 1, shellacked with a thin, fairly clear shellac. Tracing 2, shellacked with a heavier fairly clear shellac. Tracing 3, shellacked with shellac made according to the formula.

Fig. 2.—Grade F No. 5 AZO exposed for thirty seconds and developed for 1.5 minutes. Tracing 1 shows a thin shellac does not render the kymograph record paper suitable for good reproduction with average exposure and development.

The method depends upon the fact that when a tracing is made on a properly smoked drum paper, and is carefully fixed with a suitable shellac, the

<sup>2</sup>From the Department of Physiology, The Johns Hopkins Medical School.  
Received for publication, January 3, 1934.

record may be used directly as a negative, and contact prints may be made therefrom.

An outline of the complete procedure follows:

1. *Smoking the Drum Paper*.—The paper should be smoked evenly and densely, the usual care being taken not to char the paper. A yellow Bunsen burner flame is very satisfactory.

2. *Shellacking the Tracing*.—It is essential for good copies that the proper grade of shellac be used. Reference to Tracing 1 in Fig. 1 will show the reason for this. The tracing should be passed through the shellac slowly and evenly, but only once. In our laboratories we have found the following shellac to give excellent results: Enough white granular shellac to occupy one-third of an eight-liter bottle is shaken with 250 c.c. of floor varnish and with 5,084 c.c. of 96 per cent grain alcohol and allowed to stand in a warm place for a month with occasional shaking. The clear liquid is then decanted and 5 c.c. of Venetian turpentine and 100 gm. of gum mastic are added, the mixture is shaken, allowed to stand, and the clear portion is siphoned off for use.

3. *Printing the Duplicates*.—See Table I for choice of paper. Any type of printer may be adapted. A very simple one may be made by placing a light

TABLE I

USE	NAME OF PAPER	EXPOSE*	DEVELOP
For further photographic reproduction, calibration, etc. Glossy prints	Apex S No. 55 <sup>1</sup>	About 2 minutes	About 2 minutes
	Grade F. No. 5 AZO <sup>2</sup>	About 30 seconds	1.5 to 2 minutes
For notebook and classroom use where glossy surface is not necessary	Portrait proofing paper <sup>2</sup>	Less than 1 second	10 to 15 seconds
For enlarging from tracings	Apex S No. 55 <sup>1</sup>	About 4 minutes	About 3 minutes
	PMC Bromide No. 10 Glossy—extra contrast <sup>2</sup>	About 2 minutes	About 2 minutes

\*Exposure times stated are for average tracing such as Tracing 3 in Fig. 1, with a 60 watt lamp (frosted) about three inches from tracing and paper.

†Will vary with type of projector used. Two to three minutes is average with 500 watt lamp.

<sup>1</sup>Defender Film, Du Pont Film Manufacturing Co., New York City.

<sup>2</sup>Eastman Kodak Co., Rochester, New York.

inside of a box with a plate glass top. The tracing is placed in the printer with the smoked side toward the light, and the sensitized paper is placed on top of the tracing, with the emulsion side also toward the light (see Table I for exposure and development time).

4. *Development*.—The paper is developed according to the type chosen. For the types of paper listed I have found the Elon-Quinol developer, put up in tubes by the Eastman Kodak Company, to be very satisfactory.

5. *Fixing*.—All papers should be fixed for at least twenty minutes in the usual acid hypo bath.

6. *Washing of Prints*.—All prints should be washed for at least three-quarters of an hour in running water or in several changes of clean water.

The procedure as outlined is for the quick and inexpensive duplicating of records. Should facsimile duplicates be desired, a negative must first be

made on any good orthochromatic film, or a direct positive paper may be used. This, of course, adds to the time and expense necessary for turning out the duplicates.

#### SUMMARY

It is often desirable to have duplicates of kymograph tracings for calibration. A method is described whereby photographic duplicates may be easily obtained without the use of film, plate, or rephotographing camera.

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### ON THE TECHNIC OF MEULENGRACHT'S ICTERIC INDEX DETERMINATION\*

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F. BREH, PH.D., DETROIT, MICH.

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WHEN serum is diluted with 0.9 per cent sodium chloride solution, turbidity occurs rather frequently and complicates accurate comparison of the serum and standard bichromate solution.

Although determination of the icteric index is a very common procedure, little mention has been made of this difficulty. Reinhold<sup>1</sup> claims that clearer solutions are obtained if a phosphate buffer solution of pH 7 is used as a diluent. The writer finds this a valuable improvement. Perhaps an even simpler method of overcoming the difficulty consists of diluting the serum with distilled water and adding one drop of a 10 per cent solution of sodium hydroxide.

Adding a similar amount of sodium hydroxide to acetone solutions of bilirubin markedly alters their color, but no similar change occurs in the case of serum, probably because buffers are present and other conditions are different. In a series of determinations the results were identical whether sodium chloride solution, phosphate buffer solution of pH 7, or distilled water and sodium hydroxide solution in the above amount was used as diluent. The slight modification which is suggested in the test has been found useful in numerous routine determinations of the icteric index and is, therefore, communicated to other laboratories.

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\*From the Department of Laboratories, Henry Ford Hospital.  
Received for publication, February 12, 1934.

<sup>1</sup>Cited by Hawk and Bergheim: *Practical Physiological Chemistry*, ed. 10, p. 446.

# NOTES ON THE APPLICATION OF BISMUTH SULPHITE MEDIUM TO THE ISOLATION OF *B. TYPHOSUS* FROM FECES\*

T. F. SELLERS, M.D., JANIE F. MORRIS, A.B., AND MADGE REYNOLDS, B.S.,  
ATLANTA, GA.

AN INVESTIGATION of Wilson and Blair's bismuth sulphite medium at the laboratories of the Georgia State Board of Health indicates that this medium promises to be a most valuable adjunct in the work of isolating *B. typhosus* from feces for purposes of diagnosis and of detection of carriers. We have been able to find little or no reference to this medium by American investigators. We are, therefore, citing our experience with it for the benefit of those who may be interested in giving it further trial.

Wilson found in 1923 and 1926 that "*B. typhosus* in the presence of a fermentable carbohydrate is able to reduce a sulphite to a sulphide and that a combination of bismuth and sodium sulphite affords an enrichment and selective medium for *B. typhosus* and at the same time partially or completely suppresses the growth of *B. coli*."

Wilson and Blair in 1927<sup>1</sup> (see also 1931)<sup>2, 3</sup> incorporated this principle in a solid agar medium. While this medium has since undergone certain modifications, we have employed so far the original "Old Standard" formula as follows:

## 1. Stock Agar Base.—

Shredded agar (A.H.T.40)	30 grams
Peptone (Bacto or Fairchild)	10 grams
Meat extract (Liebig or Armour)	5 grams
NaCl	5 grams
Water	1,000 c.c.

Prepare in the usual way.

Note: No adjustment of the reaction is necessary.

2. *Bismuth Liquor*.—A glass stoppered bottle is almost filled with 500 c.c. water and the fluid level is marked on the side of the bottle. After pouring out the water, 60 gm. of bismuth citrate is introduced, followed with 50 c.c. of distilled water. By means of a glass rod the citrate is made into a thin paste with the water; 20 c.c. strong ammonia water is then added and stirring is continued. The resulting chemical reaction produces some heat. The glass stopper is replaced and the bottle shaken until the bismuth is almost completely dissolved.

Note: The formula calls for ammonia water of specific gravity 0.880. We found that 20 c.c. of the ammonia water we used was insufficient, but by adding more, a few drops at a time, a satisfactory solution was finally obtained. This was not water clear but had a smoky appearance.

After dissolving the bismuth, the bottle is filled up to the 500 c.c. mark. The final appearance of the bismuth liquor is slightly opalescent and upon long standing a fine precipitate settles out. This should be shaken up before using.

\*From the Laboratories of the Georgia State Board of Health.  
Received for publication, February 9, 1934.

### 3. Preparation of Medium.—

(a) To each 100 c.c. of melted stock agar add:

- 5 c.c. of 20% solution of dextrose
- 10 c.c. of 20% solution of sodium sulphite (dried)
- 5 c.c. of the bismuth liquor.

(b) Immerse the container in boiling water for two minutes, remove and add:

- 1 gram of di-sodium phosphate (anhydrous) which has previously been dissolved in 10 c.c. boiling water.
- 1 c.c. of an 8 per cent solution of ferrous sulphate anhydrous.
- 0.5 c.c. of a 1 per cent aqueous solution of brilliant green.

(c) After thoroughly mixing, pour at once into sterile Petri dishes about 10 to 15 c.c. to each dish. Allow to harden and cool with the cover ajar. Then replace covers and store in the ice box or use at once as required.

Note: The following chemical reagents used by us seemed satisfactory:

- Ammonium hydroxide—C.P. Sp. Grav. 0.90.
- Sodium sulphite, dried  $\text{Na}_2\text{SO}_3$ , Baker.
- Di-sodium phosphate anhydrous  $\text{Na}_2\text{HPO}_4$ , Baker.
- Bismuth citrate U.S.P. Merck.
- Brilliant green, Gruebler.
- Ferrous sulphate, anhydrous, Baker.

The finished product after cooling in the plates is of a pale greenish gray color, semi-opaque, and of a flaky texture, although the surface is smooth.

*Inoculation of Plates.*—Since most strains of *B. coli* are almost completely suppressed, the plates may be inoculated much more heavily than when using endo or eosin methylene blue plates. Three or four loops may be spread over each plate, taking care not to tear up the surface.

While growth of *B. typhosus* and certain other organisms may be visible after twenty-four hours' incubation, the colonies are more sharply differentiated after forty-eight hours. In the absence of *B. typhosus*, plates planted with feces are usually completely blank after forty-eight hours and can be discarded at a glance. Occasionally growth of organisms other than *B. typhosus* (see below) will occur, but it is seldom that these need be confused with that of the latter.

*The typhoid colony* when well isolated appears after forty-eight hours as rather small dry flat and very black with a metallic luster. It is surrounded by a blackish smoky halo of from one to three mm. radius. In areas of the plate where the colonies are quite close together the description as given above does not apply so constantly and the halo effect may not appear.

In Table I the growth and description of colonies of the important members of the enteric group is given. In the first column are the observations made by Wilson and Blair.<sup>2</sup> In the second column are listed the observations made by us in our own experience. Both observations were made after forty-eight hours' incubation of the plates.

Of the organisms listed in Table I, the growth and colony formation of *B. typhosus* is by far the most characteristic. On the other hand, in culturing fecal specimens on bismuth sulphite medium not every colony that is metallic black flat and dry and surrounded with a dark halo proves to be *B. typhosus*. We have occasionally encountered a variety of organisms which are capable

TABLE I  
BISMUTH SULPHITE MEDIUM

ORGANISM	DESCRIPTION OF GROWTH AND COLONIES AS NOTED BY WILSON AND BLAIR <sup>2</sup>	DESCRIPTION OF GROWTH AND COLONIES AS NOTED BY THE AUTHORS
<i>B. coli communis</i>	No growth	No growth
<i>B. coli communior</i>	No growth	Not tested
<i>B. Aerogenes</i>	A few sticky colonies	Sticky moist elevated grayish colonies with light centers—no halo
<i>B. morgan</i> No. 1	Fine greenish colonies	Small pale green colonies
<i>B. Alcaligenes</i>	No growth	No growth
<i>B. dysenteriae</i> Flexner Shiga, Hiss Y	No growth	No growth
<i>B. paratyphosus</i> A	Clear dry colonies	Medium-sized light green colonies with darker centers
<i>B. paratyphosus</i> B	Large blackish colonies	Moist grayish brown confluent colonies
<i>B. typhosus</i>	Black metallic colonies with darkening of surrounding media	Black flat dry colonies with metallic dark halo

of producing colonies very closely resembling those produced by *B. typhosus*. As yet no attempt has been made by us to identify these except to satisfy ourselves that they were not *B. typhosus*. Wilson and Blair<sup>2</sup> found that at times *B. paratyphosus* B produces colonies similar to *B. typhosus*, as do also certain sulphite reducing strains of *B. coli*. The latter, however, are usually inhibited in growth by the addition of brilliant green. We propose to investigate these later. Such "false alarm" colonies are only occasionally encountered and can easily be ruled out. However, presumptive tests such as direct slide agglutination should not be relied upon as confirmation of *B. typhosus*.

All suspicious colonies are picked and transferred to Russell's double sugar tubes and to glucose, mannose, maltose, saccharose and lactose tubes. It is often advisable to use every differentiating device available before making a definitely positive identification of *B. typhosus*.

In Table II is shown a comparison of endo and bismuth sulphite media when plated with feces from known typhoid carriers.

TABLE II

KNOWN <i>B. typhosus</i> CARRIERS	ENDO PLATES	BISMUTH SULPHITE PLATES
Mrs. S.	Pure culture typical typhoid about 300 colonies	Pure culture—innumerable typical typhoid colonies
J. F.	6 typical typhoid colonies; numerous colonies <i>B. coli</i>	75 typical typhoid colonies; no <i>B. coli</i>
E. L.	About 100 typical typhoid; numerous colonies <i>B. coli</i>	Pure culture innumerable typical typhoid colonies
T. G.	6 typical typhoid colonies; numerous <i>B. coli</i>	Pure culture, about 100 typical typhoid colonies

In all four cases the growth of *B. coli* was entirely inhibited on the bismuth sulphite plates, but in three cases the growth of *B. coli* on the endo plates greatly interfered with the detection of typhoid colonies. The endo plates were inoculated with only a fraction of a loop of specimen, while the bismuth



sulphite plates were more heavily inoculated with four full loops of specimen.

In Table III the comparative results with endo and bismuth sulphite plates obtained from a series of selected routine fecal specimens is shown.

TABLE III

FECAL SPECIMEN NO.	ENDO PLATES	WILSON AND BLAIR'S BISMUTH SULPHITE PLATES
30	Numerous <i>B. coli</i> colonies, no typhoid	Many moist green colonies, no typhoid
31	Numerous <i>B. coli</i> colonies, no typhoid	No growth
1	Numerous <i>B. coli</i> colonies, no typhoid	Numerous small black colonies, no typhoid
2	Numerous typical typhoid colonies—and a few <i>B. coli</i> colonies	Innumerable typical typhoid colonies, no <i>B. coli</i>
3	Innumerable colonies of <i>B. coli</i> and related organisms—no typical typhoid colonies observed	30 typical typhoid colonies, numerous small light brown colonies, no <i>B. coli</i>
4	Numerous <i>B. coli</i> colonies	Many colonies suggestive of <i>B. aerogenes</i> , no typhoid
5	65 <i>B. coli</i>	Two small black colonies without halos, not typhoid
6	Numerous <i>B. coli</i>	Many black colonies without halos, not typhoid
101	100 <i>B. coli</i>	150 small black colonies without halos, not typhoid
22	250 <i>B. coli</i>	100 small black colonies without halos, not typhoid
8	50 typical typhoid and 30 <i>B. coli</i> colonies	200 typical typhoid colonies, pure culture
9	15 typical typhoid, 100 <i>B. coli</i>	About 300 typical typhoid, pure culture
10	75 typical typhoid, 100 <i>B. coli</i> colonies	200 typical typhoid colonies, pure culture
13	3 typical typhoid, numerous <i>B. coli</i>	Numerous typical typhoid colonies, pure culture
11	Numerous <i>B. coli</i> , no typhoid colonies seen	2 typical typhoid, 4 unidentified colonies
111	Numerous <i>B. coli</i>	About 300 small green colonies later found to be <i>B. morgan</i>
44	Numerous <i>B. coli</i>	No growth
222	Numerous <i>B. coli</i>	No growth
1111	50 <i>B. coli</i> , 6 <i>B. alcaligenes</i>	5 black colonies not typhoid
7	Numerous <i>B. coli</i> , no typhoid seen	Numerous typical typhoid colonies pure culture
88	Numerous large clear colonies resembling <i>B. alcaligenes</i> . Also numerous <i>B. coli</i>	100 typical typhoid, pure culture
99	Numerous <i>B. coli</i> , several clear colonies suggestive of <i>B. alcaligenes</i>	1 black colony with small halo but not typhoid. 1 large black colony without halo, not typhoid. 3 round black moist colonies, not typhoid

Of the 22 specimens listed in Table III, nine proved to contain *B. typhosus* with the bismuth sulphite medium of which only 6 were positive on the endo plates. In no instance was *B. typhosus* found on endo plates and not on bismuth sulphite. Some of these specimens were from known chronic carriers, others from convalescents and some from active cases. The finding of typhoid bacilli in Specimens 11, 3, 7, and 88 would very probably have been missed if endo medium only had been used.

As previously stated, not all typhoid-like colonies appearing after forty-eight hours on bismuth sulphite plates prove to be *B. typhosus*. Therefore the usual confirmation tests should be applied.

#### ADVANTAGES AND DISADVANTAGES OF BISMUTH SULPHITE MEDIUM

##### *Advantages.*—

1. Since *B. coli* is almost completely inhibited, colonies of *B. typhosus* have room to develop. The interference of *B. coli* growth on endo is often a serious handicap in the finding of typhoid colonies, especially if the latter are not numerous.

2. Bismuth sulphite plates may be heavily inoculated without fear of overgrowth. Obviously this enhances the prospect of finding *B. typhosus*. In examining large groups of food handlers, this is particularly advantageous, in that only one bismuth sulphite plate need be inoculated. In using endo plates it is necessary to inoculate several at once or one or two daily for three or more successive days, a procedure which requires considerable time and materials.

3. *B. morgan* No. 1 grows well on bismuth sulphite plates, producing rather characteristic green colonies. Our experience with this organism suggests that its pathogenic significance has not heretofore been appreciated. Morgans' bacillus also grows well on endo, producing clear colonies not unlike typhoid. However, it is easily overlooked on endo plates, due to overgrowth of *B. coli* and to the small amount of specimen inoculated.

##### *Disadvantages.*—

1. Bismuth sulphite plates require forty-eight hours' incubation before satisfactory observations can be made. Endo plates, on the other hand, may be read after twenty-four hours' incubation.

2. Bismuth sulphite plates, in our experience, may deteriorate after three or four days even at ice box temperature. *B. typhosus* will grow to some extent on old plates, but the colonies are not always characteristic. Thus it is not feasible to prepare, as in the case of endo, a supply of poured plates larger than the anticipated needs for three or four days.

3. Bismuth sulphite medium is not so satisfactory for the culturing of the paratyphoids and dysentery bacilli, with the exception of Morgan's bacillus which probably should not be classified with the *B. dysentery* group.

4. Bismuth sulphite medium is more difficult to prepare than endo or similar media. This fact, together with its instability after three or four days, limits its use to such laboratories as have frequent occasion to examine relatively large groups of dairy workers and food handlers for the purpose of detecting typhoid carriers.

#### CONCLUSIONS

The bismuth sulphite medium as devised by Wilson and Blair is shown to be superior to endo medium as a means of laboratory detection of *B. typhosus*.

The preparation of the medium, its practical application and its advantages and disadvantages are discussed.

Bismuth sulphite medium is especially recommended for use in state and municipal laboratories where the detection of typhoid carriers among food handlers and dairy workers is an important feature.

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### A SOLUTION PRESSURE DOME FOR THE MAMMALIAN LABORATORY\*

O. G. HARNE, AND C. EARL BUTTS, BALTIMORE, MD.

FOR several years in our mammalian laboratory we have used a portable unit containing the various salt solutions (anticoagulative and physiologic) under pressure. The system has been so satisfactory from both the standpoint of utility and ease of manipulation, that we feel it should be offered for adoption.

The availability of a sensitive, low displacement value instrument such as the Tyecos sphygmomanometer, lead immediately to its utilization for measuring those pressures which ordinarily are measured with manometers and other devices having a high displacement value. Some uses are illustrated in Figs. 3, 4, 5, and 6. For example, if during a blood pressure experiment direct readings are desired, the Tyecos gauge may be cut into the circuit for a short period by manipulating stopcock *x*, Fig. 4. The systolic and diastolic pressures may be read off while the record is being made, and each experimental factor studied, may be dealt with directly in a quantitative way. When the apparatus is used to measure urinary or salivary secretion pressure, as illustrated in Fig. 6, the clamp at *x* on the outlet *K* of the apparatus is removed and applied at *x* on the flow-cannula. The low displacement value of the system allows for quick readings after which the clamps are restored and the flow again determined. This latter convenience has been found very advantageous in student work.

The apparatus may be used in parallel with a recording tambour (see Fig. 3), in closed circuit systems. Balloons in the stomach may be inflated to any desired tension, and the activity of the stomach thereupon recorded by a standardized tambour. By this method the force of the gastric activity may be recorded, and observed directly upon the Tyecos gauge.

\*From the Department of Physiology, University of Maryland, School of Medicine.  
Received for publication, February 23, 1934.

In experiments upon intracranial pressure in dogs (see Fig. 5), the pressure dome is kept in continuous connection with the cranial cannula. The citrate pressure unit is manipulated, however, exactly as in the case of blood pressure (Fig. 4).

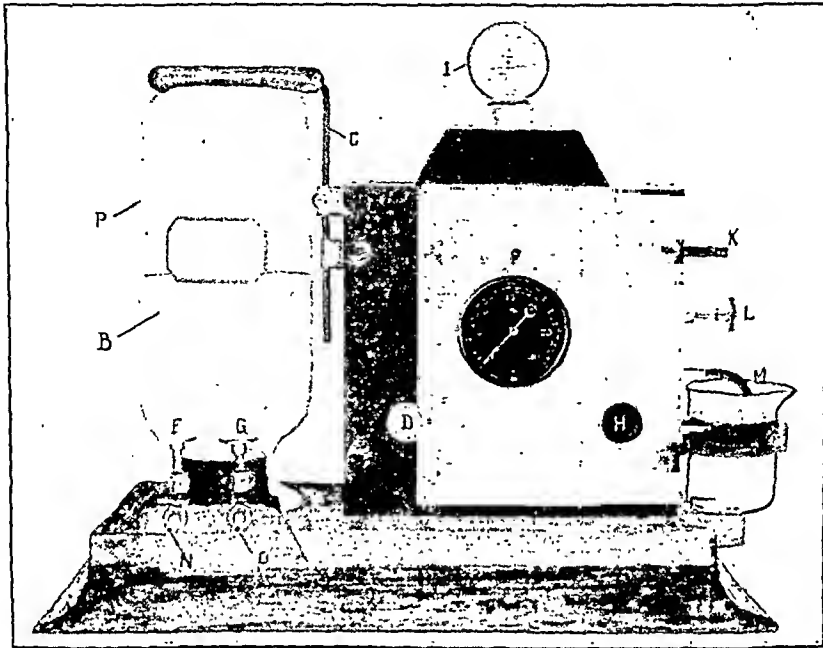


Fig. 1.—Photograph of solution pressure dome now in use. The function of each part can be understood by referring to the index letter of Fig. 2.

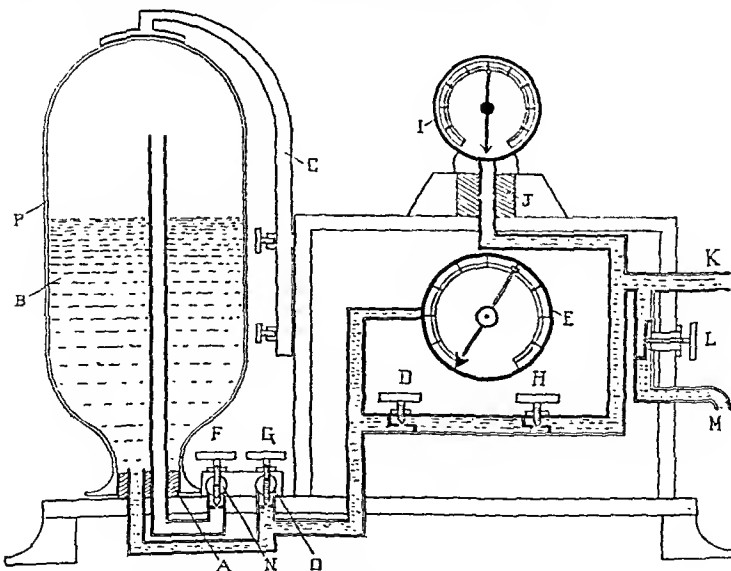


Fig. 2.—Drawing of solution pressure dome showing the parts used and their relation. For details see text.

The compressed air which is trapped in the dome is available through valve *F*, and occasionally, is found useful in clearing obstructions from cannulas

and syringe needles. These simple facilities available to the student have created a keen interest in the experiments, and have placed at his disposal means for making a number of direct observations of a fair quality of accuracy.

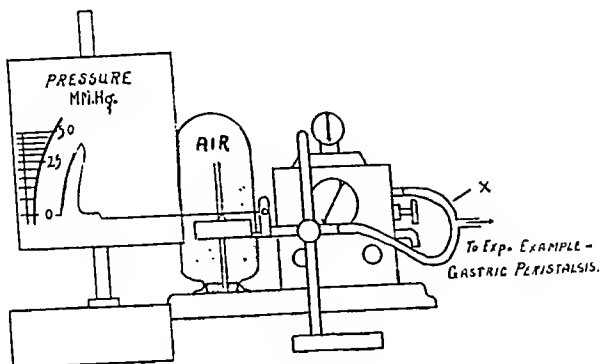


Fig. 3.—Standardizing large tambours.

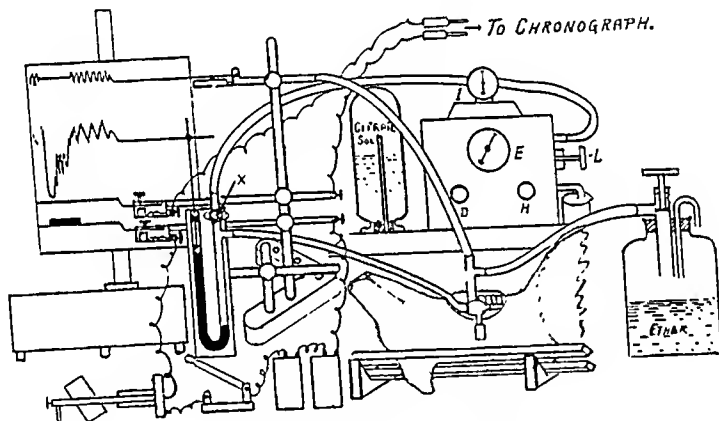


Fig. 4.—Blood pressure in dogs.

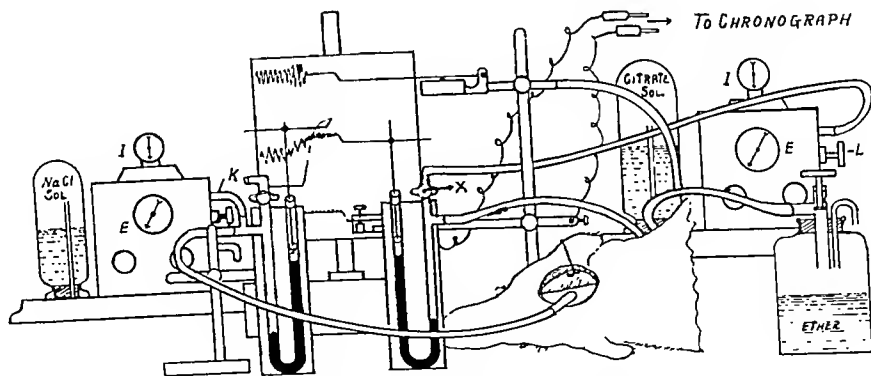


Fig. 5.—Intracranial pressure in dogs.

*The Apparatus.*—Figs. 1 and 2 illustrate the apparatus photographed, and in cross-section, respectively. The units used are easily assembled in any laboratory shop, and are available on the open market. They are: 1 two-liter

museum jar; 5 small needle valves ( $\frac{1}{8}$  inch) or better, small valves made from compression couplings; 1 small steam gauge (3-inch face); 1 Tycoos blood pressure gauge (taken from stock); 1 Tycoos pump; the necessary pipe and couplings (obtainable from any plumbing supply house).

The entire unit costs but a few dollars and can be arranged neatly and mounted as desired. The apparatus as shown in Fig. 1 is mounted to take up very little table space, and to contain an ample charge of solution for average need. The jar (*P*) is charged with 1 liter of solution through intake (*O*) of valve (*G*). Above the solution a one-liter space serves as a compression chamber. The pressure in this chamber is built up to the desired point (about 6 pounds) through connection (*N*) of valve (*F*) by a hand pump taken from a blood pressure outfit. The pressure is read off directly upon gauge (*E*). This pneumatic cushion serves as the motive power to drive the solution (*B*) through the

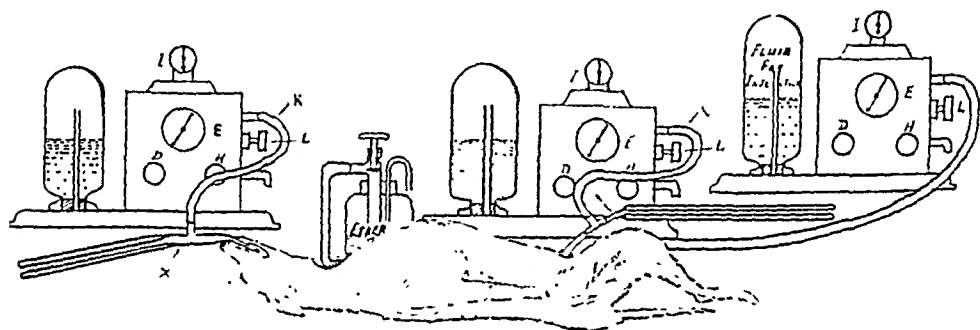


Fig. 6.—Salivary and urinary secretion pressures and fluid injections.

entire system. Two valves (*D*) and (*H*) in series provide convenient manipulation and control of the solution. (Valve *D* controls the rate of flow, while valve *H* serves primarily as a check, off or on.) By the use of this series system of valves one may set valve (*D*) at any given rate of flow, and return to it again and again. Outlet (*K*) is connected to the apparatus or system to be operated or tested and is always in parallel with pressure gauge (*I*). Valve (*L*) is a drain, and is used to release fluid from the system or to reduce the pressure built up through valves (*D*) and (*H*). (*A*) and (*J*) are rubber stoppers, which seal the museum jar (*P*) and the Tycoos gauge mounting. By this system both the jar and the gauge may be removed instantly. The system is prepared for storage by emptying the jar and forcing through the system air under pressure. The systems come out of storage in good condition and are quickly placed into use.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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**SYPHILIS, Standard Treatment Procedure in Early: A Résumé of Modern Principles,** Stokes, J. H., Cole, H. N., Moore, J. E., O'Leary, P. A., Wile, U. J., Farran, T. Jr., Vonderlehr, R. A., and Usilton, L. J. J. A. M. A. 102: 1267, 1934.

The résumé following is the product of a massive, world-wide investigation and represents the generally acceptable principles applicable to the management of this disease.

## CONTROL OF INFECTIONOUSNESS IN SYPHILIS

1. Infectiousness in syphilis is a function of three factors: (a) time; (b) arsphenamine; (c) individual predisposition to relapse.

2. Infectiousness is not a function of the serologic state of the patient. No serologic test has any value as a proof of infectiousness or noninfectiousness, early or late.

3. Syphilis, treated or untreated, is most infectious early, grows less with lapse of time, is rarely infectious (but may be so) after five years. Late syphilids and late prenatal (congenital) cases are not infectious.

4. Therefore, spar for time and delay in any issue involving infectiousness (i.e., marriage, intercourse).

5. Infectiousness is controlled and syphilis will be extinguished, if ever, as a health problem, by the treatment of the infectious person.

6. The public health responsibility of the physician is therefore with the early months and years of the disease.

7. Treatment to control infectiousness must be with the arsphenamines. No other drug will do.

8. The use of arsphenamines must not be delayed even to secure any minor individual immunizing advantage.

9. Treatment to control infectiousness must be continuous, not intermittent, and last at least eighteen months. Rest periods encourage relapse.

10. Acetarsone (Stovarsol), tryparsamide, and nonspecific (including fever) therapy must not be expected to control infectiousness.

11. Search for infectious lesions is ineffective, except as an adjunct. Hence inspection of prostitutes is useless.

12. Instruction and cooperation by the patient is ineffective and untrustworthy except as adjunct.

13. The amount of arsphenamine required is not less than twenty injections. The critical point is between 5 and 9.

14. Heavy metal is required as an adjunct.

15. The infectiousness of semen and vaginal secretions, even in the absence of lesions, in early and latent syphilis demands absolute mechanical protection in intercourse, treatment or no treatment. Continence, advised, is seldom practiced.

16. Inspection, instruction, control and protection are more essential in rest periods than under treatment.

17. There is a relapsing type of early syphilis regarding which no rules or predictions can be formulated.

18. In relapsing types, infectiousness may reappear immediately after, or even during (arsphenamine resistant), treatment with the arsphenamines.

19. Alcohol, dirt, bad hygiene, irritants including tobacco, sweat, friction (intercourse) predispose to infectious lesions.

20. The great promoter and source of relapse is the *short* arsphenamine course (one to four injections) unsupported by other treatment.

21. Treatment prophylaxis (after exposure) is unreliable. If given it must be followed through.

22. Adequate examination of patients would protect physicians, dentists, nurses and transfusion recipients from most risks of accidental infection.

23. The nonpregnant, nonsyphilitic woman should be protected mechanically and by treatment of the partner.

24. Conception should not take place except under treatment control.

25. The child of the pregnant syphilitic woman should not be destroyed, but protected in utero by treatment of the mother, before and after conception, and of the father, if syphilitic, before conception.

The term applied by Pusey to the superior outlook of treatment begun in the primary stage of syphilis before the blood serologic tests become positive ("seronegative primary") has been justified abundantly by statistical analysis. The proportion of "cures" when treatment is begun in the seronegative primary stage (diagnosis by dark-field or other identification of *Spirocheta pallida*) is, as given, 71.4 per cent average and from 83 to 86 per cent best results. When, through failure of the patient to present himself or of his physician to diagnose primary syphilis until the blood test becomes positive, treatment is not begun until the so-called seropositive primary stage "cure" is attained in only 53.3 per cent by average and from 64 to 70 per cent by the best methods. This represents a clear loss of 18 per cent in outlook for "cure" by the delay. If the patient goes on to the development of a secondary eruption of course with a positive blood test, "cure" is attained in only 50 per cent by average and 61 to 82 per cent by best methods. This represents a possible loss of 21 per cent by average and 14 per cent by best methods over the outlook prevailing when treatment is begun in the seronegative primary stage.

It is accepted now as axiomatic that no patient with early syphilis should be treated by the sole use of an arsenical.

Just as no patient should be treated exclusively with an arsenical, so also no patient should be treated exclusively with a heavy metal, even with bismuth, for all that it possesses greater spirocheticidal qualities than mercury.

One of the most important contributions of recent years to the technic of treatment of early syphilis has been to the question of intermittence as distinguished from continuity of treatment.

It may now be said with positiveness that the old practice of administering treatment in early syphilis by fits and starts, conditioned on the Wassermann report of the blood, is pernicious; that even the introduction of a few weeks of complete rest from treatment into the management of the first eighteen months of the disease is likely to be profoundly injurious; that the patient who lapses or escapes treatment during this period is his own worst enemy; and that no rest intervals, and a regimen in which the patient is constantly receiving either an arsenical or a heavy metal during the first year of the disease or longer, if the indications require, is the best and safest modern practice, in the interest both of the patient and of the public health.

Continuous treatment, then, whether prolonged or brief, and practically regardless of the drugs used, is superior in its results to the intermittent or other schemes of treatment.

The failure of the blood Wassermann reaction to reverse is more a matter of how treatment is given than of how much treatment is administered. A little treatment continuously given is more than twice as effective as when intermittently applied and more than four times as effective as when irregularly given. Prolongation and intensification of treatment, however, using much arsphenamine and much heavy metal, but especially much arsphenamine in the first three months, promotes good results. Much arsphenamine and much heavy metal is four times as effective as little arsphenamine and little heavy metal in securing a negative Wassermann reaction within this period, when the drugs are continuously used.



## A SCHEME OF TREATMENT FOR EARLY SYPHILIS

DAY OR WEEK	ARSPHEN- AMINE, GM.	INTERIM TREATMENT	BLOOD WASSER- MANN REACTION	COMMENT
Day		-----	1	Arsphenamine dosage for first 3
1	0.3-0.6			injections at level of 0.1 gm. for
5	0.3-0.6			each 25 pounds body weight; 0.4
10	0.3-0.6			gm. men; 0.3 gm. women; in
				average patient all lesions heal
				rapidly and blood Wassermann
Week				reaction becomes negative dur-
3	0.4			ing first course; if arsphenamine
4	0.4			cannot be used, substitute 8 to
5	0.4			10 doses 0.3 gm. silver arsphen-
6	0.4			amine, or 10 to 12 doses 0.6 gm.
				neoarsphenamine; this applies
				also to subsequent courses.
7	0.4		1	If mercury is used note overlap of
8		Bismuth, 4 doses,		one week at end of first and
9		0.2 gm. and KI		start of second arsphenamine
10		or Ung. Hg. and		courses; at this point a few
11		KI		days without treatment may be
				dangerous; neurorecurrence.
12	0.4			Arsphenamine starts, bismuth
13	0.4			stops; watch for provocative
				Wassermann reaction after first
				dose of arsphenamine.
14	0.4			Try to prevent short lapses in
15	0.4			treatment, especially at this
16	0.4			early stage.
17	0.4			
18-23		Bismuth, 6 doses,	1	
		or Ung. Hg. and		
		KI		
24	0.4			Bismuth is better than mercury;
25	0.4			use it if possible; examine cere-
26	0.4			brospinal fluid at about this
27	0.4			time if patient's cooperation can
28	0.4			be secured.
29	0.4			
30-37		Bismuth, 8 doses,		
		or Hg. and KI		
38	0.4		1	Note that bismuth or mercury
39	0.4			courses are gradually getting
40	0.4			longer—4, 6, 8 and now 10
41	0.4			weeks.
42	0.4			
44-53		Bismuth, 10 doses,		
		or Ung. Hg. and		
		KI		
54	0.4		1	The average seropositive primary
55	0.4			or early secondary patient should
56	0.4			have at least 5 courses of
57	0.4			arsphenamine.
58	0.4			
59	0.4		1	
60-69		Bismuth, 10 doses,		It is safer to finish treatment with
		or Ung. Hg. and		bismuth or mercury rather than
		KI		with arsphenamine.
70-122		Probation. No	6-12	Blood Wassermann every month if
		treatment.		possible, at least every other
				month.
123		Complete physical and neurologic examination, spinal puncture and, if possible, fluoroscopic examination of cardiovascular stripe. Thereafter, yearly physical examination blood Wassermann every six to twelve months; if the two spinal fluid examinations above are negative, this need not be repeated.		

The distressing frequency of the practice, for which physician or patient may be to blame, of giving four or five arsphenamine injections, testing the blood, finding the patient Wassermann negative in an early case, and virtually dismissing him with a few pills, inunctions or heavy metal injections should be fought with every resource that current knowledge can bring to bear.

It appears from the Cooperative Clinical Group investigation that the original arsphenamine when used alone is superior in rapidity of action on the blood Wassermann reaction; but the deficiencies of neoarsphenamine are to some extent compensated for by its use with a heavy metal and by its employment in a continuous rather than an intermittent system of treatment.

It appears that after two years of observation or treatment the largest number of patients obtaining satisfactory results falls in the category of those receiving from twenty to twenty-nine injections of an arsphenamine and a similar amount of heavy metal. Thus, thirty injections of the arsenical becomes a therapeutic objective, in place of the "forty or over" suggested by the results of earlier investigators.

#### REACTION PREVENTION PRINCIPLES

1. Inquire into the history of idiosyncrasy, allergic tendencies, skin irritability (especially eczema and seborrhea), focal and intercurrent infection, liver damage, and pregnancy before treatment is begun.

2. Question the patient before each treatment regarding (a) itching skin or rash; (b) purpura and melena; (c) gastrointestinal reaction; (d) condition of the mouth and teeth.

3. Examine at least the eyes (jaundice), face (dermatitis), mouth (salivation, bismuth pigment, purpura), flexures of the elbows (dermatitis), wrists and ankles (purpura) before each treatment. Take the temperature.

4. Make the first dose of any drug not more than half the full dose.

5. Pull back on syringe pistons: before intravenous injections to be sure of vein entry; before an intramuscular injection to be certain a deep vessel has not been entered.

6. Inject intramuscularly into the inner angle of the upper outer quadrant of the buttock and massage long and well after the injection.

7. Inject all solutions for intravenous use slowly through a small needle; not faster than 0.1 gm. per minute for neoarsphenamine.

8. Keep carbohydrate and alcohol low in the diet, and protein and fat high.

9. Permit only a light meal before and after an arsenical and prescribe a mild cathartic the morning after.

10. Make a urine examination biweekly.

11. Give calcium freely.

#### PHYSICIAN-PATIENT RELATIONSHIP AND RESPONSIBILITY

The physician should lay before the patient the facts under these heads:

1. That he has syphilis and present the evidence for the statement.

2. His outlook for "cure" with emphasis on the excellent reward of persistence in early cases.

3. The general facts of infectiousness as previously outlined.

4. The possibility of marriage, under medical control with personal cooperation, and the possibility of healthy offspring under prenatal direction and treatment.

5. The necessity for postponement or avoidance of marriage, intercourse, and pregnancy until the infection is under therapeutic control with the arsphenamines.

6. The relative inefficiency of the blood serologic tests as a measure of infectiousness, fitness for marriage or conception and "cure" (reiterate).

7. The dangers (relapse, neurorecurrence, serologic irreversibility and treatment-fastness, precocious tertiarism and malignant syphilis) of inadequate, short course, and irregular treatment.

8. The lulling into false security produced by the quick disappearance of symptoms under treatment.

9. The probable treatment requirements; including at least an eighteen months' estimate for early syphilis, whether seropositive or seronegative, primary or secondary.

10. Personal hygiene, control of treatment reactions, and symptoms of infectious recurrence.

11. The need for observation throughout life.

12. Special arrangements (examination of contacts and infection source; of family; communication of facts to others; treatment arrangements for travelers and nonresidents).

13. The probable costs and their settlement or adjustment.

**TUBERCLE BACILLI, in the Gastric Contents of Tuberculous Children, Gourley, I. Am. Rev. Tuberc. 29: 461, 1934.**

Fifty-nine cases of childhood tuberculosis have been studied by gastric lavage and guinea pig inoculation. All of these children reacted positively to the intracutaneous tuberculin test.

Fifty of the 59 patients had demonstrable lesions in the roentgenograms of the lungs. Nine had no demonstrable lesions. Their only evidence of tuberculosis was the positive tuberculin test.

Twenty-eight cases, 47.4 per cent, of the 59 were found to have tubercle bacilli in the gastric contents. Thirty-one cases, or 52.6 per cent, of the 59 cases studied, were negative for tubercle bacilli in the gastric contents. Of the 50 children with demonstrable lesions, 28, or 56 per cent, had positive gastric contents.

Fourteen children, or 50 per cent of the cases with positive gastric contents, were of school age. Should children with gastric contents containing tubercle bacilli be kept from close contact with healthy children?

Twelve children had calcium in the parenchyma of the lungs or regional lymph nodes. Five of these had positive gastric contents.

This study apparently indicates that all types of parenchymal lesions in childhood tuberculosis including the "C" group of McPhedran's classification are open cases. Further study is to be made before a definite conclusion can be reached.

**TUBERCLE BACILLI, Demonstration of, in the Feces, Sputum, and Stomach Contents of Tuberculous Children, Mishulow, L., Kereszturi, C., and Hauptman, D. Am. Rev. Tuberc. 29: 471, 1934.**

From the results obtained in this investigation, the following conclusions may be drawn:

1. Guinea pig inoculation should be made as a final confirmation when specimens are negative on smear examination.

2. Repeated examinations should be made in doubtful cases, inasmuch as positive and negative results are frequently obtained in the same case when the specimens are taken on different days.

3. The indications are that repeated examinations of feces by smear and guinea pig inoculation may prove as satisfactory as the examination of gastric lavage specimens.

4. As a diagnostic method, the examination of feces would be much more desirable than gastric lavage, as it could be easily repeated as often as necessary without any discomfort to the patient. Although gastric lavage examination has given very satisfactory results it is not always a desirable procedure, as it is hard on the patient when repeated examinations are made, and it is not feasible in some cases, especially when the patient is not hospitalized.

5. In the 60 cases in this series, the examination of feces, gastric contents, and sputa (when they were obtained) showed 10 per cent positive in children who were apparently well at the time of examination, 54.5 per cent positive in those that were slightly ill, and 63.1 per cent positive in the children who were very ill.

6. Of the children who had destructive parenchymatous pulmonary lesions, 83.3 per cent showed positive results and of those who had nondestructive parenchymatous lesions, 24

per cent were positive. Cases with only positive intracutaneous tests, hilum lymph node tuberculosis, pleurisy with effusion, and tuberculous peritonitis, were all negative.

**TUBERCLE BACILLI, In the Sputum and Feces of Children Without Pulmonary Tuberculosis, Nalbant, J. P.** *Am. Rev. Tuberc.* 29: 481, 1934.

Out of 19 children under fifteen years of age, 7 were found to have tubercle bacilli in the contents of their gastrointestinal tract. They had no demonstrable pulmonary tuberculosis.

**BLOOD SEDIMENTATION RATE, Relationship Between Fibrinogen Content of Plasma and, Gilligan, D. R., and Ernstene, A. C.** *Am. J. M. Sc.* 187: 552, 1934.

One hundred and ninety simultaneous measurements of the fibrinogen content of the plasma and the "corrected sedimentation index" were made in normal individuals and in patients with various kinds and degrees of pathologic conditions.

A close correlation was observed between the plasma fibrinogen content and the corrected sedimentation index.

In certain cases with liver damage the corrected sedimentation index is increased disproportionately to the increase in plasma fibrinogen content.

The results of the investigation indicate that, except in certain cases with liver damage, the plasma fibrinogen content plays the major rôle in controlling the corrected sedimentation index.

**LEUKOCYTE COUNT, Effect of Hyperpyrexia Induced by Radiation Upon the, Bierman, W.** *Am. J. M. Sc.* 187: 545, 1934.

Hourly observations were made of the changes occurring in the leucocyte count of patients suffering from varied diseases in whom hyperpyrexia was induced by means of radio waves of 30-meter length.

An initial reduction, about 25 to 30 per cent, in the number of leucocytes regularly occurs, usually during the first or second hour of treatment. This is constantly followed by a leucocytosis whose maximum, amounting to about 80 per cent above the initial figure, occurs about the sixth to the ninth hour.

These variations are due mainly to changes in the total number of neutrophils, of which the staff neutrophils show the greatest increase. These changes, together with the appearance of other immature forms, indicate a stimulation of the bone marrow.

At a later stage the monocytes and lymphocytes also increase in number.

Repeated stimulation by heat is followed by a reduction in the leucocyte response.

**ARTHRITIS, Rheumatoid, Autogenous Vaccines in, Short, C. L., Dienes, L., and Bauer, W.** *Am. J. M. Sc.* 187: 615, 1934.

Skin tests made with bacterial strains isolated from arthritic patients do not allow us to select specific strains for vaccine therapy.

Skin tests without using several subjects as controls are without any significance.

Variations in the skin reactions may be explained by differing irritability of the patient's skins, natural toxicity of the bacterial species, or possibly by a sensitization to certain bacterial groups.

The different methods which have been recommended for the selection of specific vaccine strains are without a solid theoretical or experimental foundation.

The above conclusions suggest that the therapeutic effect, if any, gained from autogenous vaccines is nonspecific.

The hypothesis that rheumatoid arthritis is a disease of allergic origin is not supported by conclusions drawn from the interpretation of uncontrolled skin reactions with autogenous vaccines.

**LEUKOCYTE COUNT, Following Obstetric Analgesia Produced by Pentobarbital Sodium,**  
Hardwick, R. S., and Randall, L. M. J. A. M. A. 102: 1558, 1934.

There has been nothing in the behavior of the mother or infant in any case in which pentobarbital sodium was administered to indicate any deleterious effect. Studies of the blood from this group of patients give no evidence of the production of a leucopenic condition with the amounts of pentobarbital sodium administered.

This series of studies of leucocyto and differential counts indicates that the greatest leucocytosis occurs at the fifth hour postpartum. The level of leucocytes falls steadily to the fourth day, when the number of leucocytes per cubic millimeter of the blood remains constant until the last examination on the tenth day.

**TAKA-ARATA TEST, A Modified Mercuric Chlorid Reaction in Cirrhosis and Neoplasms of the Liver,** Crane, M. P. Am. J. M. Sc. 187: 705, 1934.

*Method.*—Reagents: 0.9 per cent sodium chloride solution; 10 per cent sodium carbonate solution; 0.5 per cent mercuric chloride solution; 0.025 per cent aqueous fuchsin solution.

Into each of a row of 8 small glass tubes (those used for determining blood hemolysis are quite suitable) is placed 1 c.c. of 0.9 per cent sodium chlorid solution. To the first tube is added 1 c.c. of blood serum, ascitic or other fluid. From the mixed content of the first tube 1 c.c. is transferred to the second, the tube shaken, then 1 c.c. transferred from Tube 2 to Tube 3, and this procedure is continued throughout the series. In the eighth tube the 1 c.c. removed is discarded. The dilution of the serum or other fluid then ranges from 1 to 2 in the first tube to 1 to 256 in the last. To each tube is then added 0.25 c.c. of the sodium carbonate solution and 0.3 c.c. of the freshly prepared Takata reagent, which consists of a mixture of equal parts of the mercuric chlorid and aqueous fuchsin solutions. Readings are made immediately, and at one-half-hour and twenty-four-hour intervals. They depend entirely on the precipitate in the tubes. The precipitate at the end of twenty-four hours is almost invariably the most definite and constant, since precipitate which appears at earlier readings frequently diminishes or disappears on standing. Clouding and color changes are without importance; the same is true of the blue granular precipitate that sometimes occurs in the tubes of highest dilution, i.e., the seventh and eighth.

In view of the fact that we are concerned only with precipitation and that color changes play no part, the author has found it possible to omit the fuchsin. A further slight modification is the use of 6 tubes, rather than 8; for as previously mentioned, the precipitate in the last 2 tubes is without significance, and often occurs in distilled water alone.

The readings are made as minus (no precipitate, 1+, 2+, or 3+). Charting is of convenience. After moderate practice, charting is unnecessary and readings are negative, positive, or, for the sake of comparison in cases that are followed progressively, strongly positive.

The standard by which positive reactions are judged is the appearance of a definite precipitate in 2 of the first 3 tubes, and at the same time a precipitate in any number of the following tube, with the exceptions as previously noted. Positive reactions are usually quite characteristic, and negatives may have no precipitate in any tube, or a 1+ or a 2+ in any of the tubes from 4 to 7 alone.

Blood if freshly collected must be centrifuged; due care must be exercised to prevent hemolysis, as it is possible that this may cause false positives. The presence of bile is without influence. Blood or other fluids may be preserved almost indefinitely on ice or with toluol. Ascitic fluid that contains blood or pus must also be centrifuged.

The test is positive only in advanced cirrhosis, and is therefore of little value from the standpoint of early diagnosis.

Its chief worth lies in its differential diagnostic value to distinguish between cirrhosis and various liver neoplasms and other advanced liver disturbances.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

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### Recent Advances in Sex and Reproductive Physiology\*

PROFESSOR F. A. E. CREW, in the introduction to this book remarks that: "Man has turned from the adventurous conquest of his environment to the conquest of himself. Today is the day of biological invention, eagerly used for the control of the undesirable and the unwanted. Sex and reproduction are no longer hedged around by myth and taboo; they are no longer accepted as mysteries that defy understanding. They are matters inviting examination and explanation; they are regarded as expressions of physico-chemical forces, the nature of which is to be displayed. It is accepted that when knowledge is sufficient, control will be absolute, and, though knowledge is not yet sufficient, readers of this book must be persuaded to the view that this will not always be so."

The purpose of the volume is to present to the reader a survey of the present status of investigations concerned with the sex glands and the female sex hormones. As, of course, the greater part of the available information in this field is the result of animal research the work is naturally concerned with the studies made on animals and especially with those conducted on primates.

The volume deals essentially with the sexual and reproductive phenomena in the female in relation to the activity of the sex hormones.

It is an easy reading compilation and correlation of the work of many investigators which will serve the purpose of concentrating under one cover much of the literature reporting the investigations in this field.

A fairly extensive bibliography is appended which does not include, however, many American references of interest to students of this problem.

The book can be recommended to all who are interested in this phase of medical investigations.

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### Neuropathology†

THE development of neurology as an independent unit of the medical sciences, and the great advance which has been made in this highly specialized field has naturally been accompanied by a corresponding accumulation of facts concerning neuropathology, also a highly specialized subject.

Dr. Weil's book, therefore, should be welcomed, not only by the neurologist and neuropathologist, but also by the practitioner at large as a ready source of reference.

The purpose of the book is to present a review of the present status of neuropathology which is achieved in a very comprehensive and satisfactory manner.

The comprehensive character of the discussion is indicated by the Chapter headings which follow: Changes Through Autolysis and Fixation; Diseases of the Ganglion Cells; The Glia and Its Pathology; Pathology of the Myelin Sheaths and the Axis Cylinder; Anemic Softening; Arteriosclerosis; Inflammation; Infections; Intoxications; Injuries; Degenerations; Tumors; and Congenital Malformations.

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\*Recent Advances in Sex and Reproductive Physiology. By J. M. Robson, Research Fellow, Institute of Animal Genetics, University of Edinburgh. Cloth, pp. 249, 47 illustrations. P. Blakiston's Son and Co., Philadelphia, Pa.

†A Textbook of Neuropathology. By Arthur Weil, M.D., Associate Professor of Neuropathology, Northwestern University Medical School. Cloth, pp. 335, 260 illustrations. Lea and Febiger, Philadelphia.

An appendix contains a description of satisfactory methods for the autopsy and fixation of the central nervous system.

It is not to be expected that the perusal of this book will make of the reader a neuropathologist. The clinician may well refer to it for a better understanding of the relationship of the nervous system to disease in general; the pathologist will find it a useful and valuable reference; and to the neuropathologist it presents under one cover a succinct yet thorough survey of the information at present available.

The book evidences a wide experience and a thorough acquaintance with the subject and can be well recommended to those who are, or should be, interested in this subject.

The illustrations are not only numerous but excellently reproduced. An extensive bibliography and satisfactory index are appended.

### The Medicolegal Necropsy\*

THIS book should be of the greatest interest, not only to coroner's physicians, pathologists, and all who may be called upon to perform necropsies for medicolegal purposes, but also to the physician at large as a reliable and comprehensive survey of a very important subject.

The introductory chapter by Dr. F. E. Sondern reviews the faults of the present coroner's system and the following chapter by Dr. O. T. Schultz outlines the workings of the medicolegal system of the United States and the status of the medicolegal autopsy.

Dr. Charles Norris, Chief Medical Examiner of New York City, furnishes a chapter on The Medicolegal Necropsy and the manner in which it should be carried out is described by Dr. A. V. St. George. Toxicology in the Medicolegal Necropsy is described by A. O. Gettler, followed by a very comprehensive discussion of the findings in deaths from shooting, stabbing, cutting, and asphyxia by Dr. H. S. Martland, Chief Medical Examiner for Essex County. E. L. Miloslavich, of the Institute of Legal Medicine of Yugoslavia contributes an excellent discussion of the pathological anatomy of death by drowning.

An appendix presents the report of a committee on necropsies which should be read by every pathologist.

The high standing of all the authors assures the reader of an authoritative presentation based on extensive experience.

This small volume may well become a standard reference text and deserves, as it will doubtless receive, a wide circulation.

It contains so much and so varied information that it might well have been furnished with an index.

The book may be highly recommended without reserve.

### Diabetic Manual for Patients†

DIABETIC manuals for the use of patients were started prior to the era of insulin, especially by Joslin. At that time the importance of carefully measured and controlled dietaries was great. Since insulin, the impression has become very general both among the laity and among physicians that as long as a patient takes his insulin he need not follow any carefully supervised dietary program. This makes for carelessness and in the end usually results in continued loss of tolerance. No one knows better than those whose special interest

\*The Medicolegal Necropsy: A Symposium Held at the Twelfth Annual Convention of the American Society of Clinical Pathologists. Edited by Thomas B. Magath. Cloth, pp. 167. 63 illustrations. Williams and Wilkins Co., Baltimore, Md.

†Diabetic Manual for Patients. By Henry J. John, M.A., M.D., F.A.C.P., M.A.J., M.R.C., Director of the Diabetic Department and Laboratories of the Cleveland Clinic. Second Edition. Cloth, pages 232, 1931. The C. V. Mosby Company, St. Louis, Mo.

is in the field of diabetes that for optimal results the patient still must know intimately the details of his disease and must carefully control his diet even though the diet is many times more generous than in the old starvation period.

The second edition of John's Manual would appear to fulfill excellently the need for which it was written. It tells the patient much about himself and goes into details such as the desirability or otherwise of marriage of diabetics, the relationship of obesity to the disease, the proper prevention of complications, acidosis, coma, gangrene and proper hygiene for the diabetic patient. The language is sufficiently simple so that any patient with a modicum of intelligence can read and understand. The illustrations are thoroughly descriptive, particularly those which accompany the food tables, and show, in red, the carbohydrate content of the various foods. This red danger signal stands out clearly with the result that the diabetic patient has no difficulty in a visual understanding of the need for avoiding this and that.

Dr. John has pioneered in the summer camps for diabetics. In discussing the value of this he emphasizes that the diabetic child is at a decided disadvantage in the summertime when other children may go to camp. And the mother is at a greater disadvantage because she must stay at home and continue to treat the child. The summer camp is a wonderful curesease for both mother and child. In the preinsulin days this was not a necessity among diabetic children because their lives were short at best. Now, when they are growing to adult life and usefulness, they must be prepared therefor.

The book should continue to fulfill in an excellent manner the purposes for which it was written.

### The Compleat Pediatrician\*

THIS is a book which can be recommended without reserve. In few specialties in the field of medicine will the physician be confronted with more diagnostic difficulties than in pediatrics for the very obvious reason that in this specialty he is to a very large extent dependent upon his powers of observation, his clinical skill in noting and eliciting the signs and symptoms of disease, and especially upon the degree to which he is prepared to utilize the processes of inferential deduction developed through reading, study, and past experience.

While, as the author comments in his preface, the best way to obtain a durable and useful knowledge of pediatrics—as well as, indeed, of any branch of medicine—is through the study of the patient, he recalls also Osler's saying that, "to study the phenomena of disease without books is to sail an uncharted sea, while to study books without patients is not to go to sea at all."

As the title page suggests, Dr. Davison set himself an ambitious and comprehensive task. Study of the book reveals that in its execution he has fallen but little, if anything, short of fulfilling his plan.

This book consists of Chapters: I, Symptoms and Signs of Disease in Children; II, Diseases, Differential Diagnosis, and Treatment; III, Preventive Measures and Child Care; IV, Administration of Fluids and Blood to Infants; V, General Suggestions for the Feeding of Normal Infants and Children; VI, Drugs and Prescriptions Frequently Used in Pediatrics; and VII, Laboratory Methods Frequently Used in Pediatrics.

An Appendix (B) presents an outline for pediatric histories and the physical examination of children, while Appendix C lists the contents of the pediatrician's bag.

While some one complained somewhere that of the making of books there is no end, Dr. Davison's book stands almost in a category by itself. Its plan is not only original but well carried out and the contents reflect not only an extensive and digested experience, but also a comprehensive acquaintance with and survey of the literature of pediatrics and may well be to the pediatrician what Roget's *Thesaurus* is to the writer.

\*The Compleat Pediatrician: Practical, Diagnostic, Therapeutic, and Preventive Pediatrics for the Use of Medical Students, Internes, General Practitioners and Pediatricians. By Wilburt C. Davison, M.D., Professor of Pediatrics, Duke University, School of Medicine. Cloth, pp. 260. Duke University Press.



The plan is that of a thesaurus permitting extensive and diversified cross reference. While the numbered paragraphs may present, to the novice, a peculiar and perhaps complicated appearance, the book is simple to use.

As always, the first requisite is a careful history and examination. Should the presenting symptom be, for example, headache, one finds this alphabetically listed in paragraph 16 of Chapter I. Under the causes, the reader is referred to the *common* causes as well as to *rare* causes, each bearing a paragraph number such as, for example, to typhoid-paratyphoid fever (851) meningococcus meningitis (531), sinusitis (775), eyestrain (359), etc. In these latter paragraphs will be found descriptions of additional signs and symptoms which should be present for diagnosis; references to other diseases which will cause similar symptoms; to various diagnostic laboratory measures; to the most useful therapeutic measures; and, finally, to any possible preventive measures.

The book, therefore, is exceedingly simple to use and remarkably and most commendably practical in the presentation of its contents. It abounds, moreover, in excellent concise tables especially in Chapter I.

The laboratory section is both comprehensive and practical as, indeed, is true of the book as a whole.

Dr. Davison is to be congratulated in having made a permanent addition to pediatric literature and one which deserves—as it will doubtless receive—an enthusiastic reception from the large and varied audience to which it is addressed.

To the laboratory worker, often called to interpret the laboratory findings in children, it should prove a most useful work of reference.

While it is questionable if there is anything in the field of pediatrics concerning which some mention will not be found in this book, emphasis is wisely laid upon the most important diseases encountered in children. Of these (as determined by incidence in 80,000 children representing 150,539 cases over a period of twenty years in the wards and dispensaries of Johns Hopkins Hospital and the Harriet Lane Home) there are 307, only 100 of which are important.

These 100 diseases cause 56 per cent of the deaths in children and can be prevented, and 21 per cent of the pediatric deaths, although responding to adequate treatment.

We repeat that this book may be recommended without reserve as an invaluable contribution to pediatric literature.

### Physical Diagnosis\*

CABOT'S *Physical Diagnosis* needs no introduction and the fact that it has now reached its eleventh edition is no surprise to those who are familiar with it—as who is not?

While it represents, as the author emphasizes and reiterates, the strictly personal viewpoint of the author, and while only that is included with which the author has had personal experience, this book is nevertheless a valuable, useful and practical contribution with which every practitioner and student of medicine should be familiar.

In thus presenting what he himself has found to be valuable in the study of disease, it is inevitable that the reader will encounter omissions, such as, for example, no mention of "poker spine" as an early sign of poliomyelitis under the discussion of "stiff back."

The book is a clearly written account of Dr. Cabot's own experience which is admittedly extensive, well told, and may well find a place in the library of every practicing physician.

\*Physical Diagnosis. By Richard Cabot, M.D., Professor of Clinical Medicine Emeritus, Harvard University. Edition 11, cloth, pp. 540, 317 figures. William Wood and Co., Baltimore, Md.

# *The Journal of Laboratory and Clinical Medicine*

VOL. 20

ST. LOUIS, MO., NOVEMBER, 1934

No. 2

Editor: WARREN T. VAUGHAN, M.D.

Richmond, Va.

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M. H. SOULE, Sc.D.	- - - - -	ANN ARBOR, MICH.

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## EDITORIALS

### **The Laboratory Diagnosis of Tuberculous Infection in Childhood**

THE essential importance of early recognition of tuberculous infection in childhood has long been recognized but the diagnostic problem presented is not always easy of solution.

Symptoms may be indefinite, the roentgenographic picture and the results of tuberculin tests not always clear-cut, and the procedure commonly applied in the adult, the examination of the sputum for tubercle bacilli, is often inapplicable either because the children do not cough, or are unable to bring up sputum, or the sputum is swallowed.

Very naturally the search for these organisms then turned to the gastric contents or the intestinal discharges, the examination of specimens secured by gastric lavage being first suggested by Meunier<sup>1</sup> as long ago as 1898. Since then the procedure has been studied somewhat spasmodically by a number of investigators with somewhat variable results but, on the whole, showing that the method was sufficiently valuable to be borne in mind in the doubtful case.

While a positive response to the intracutaneous tuberculin test is of definite significance, the important fact, as regards the arrest, control, and dissemination of tuberculosis is to determine whether or not the case is open and active.

The tuberculous child with an active and open lesion discharging tubercle bacilli is obviously more of a menace to other children around it than one in whom these circumstances do not obtain; and it is to this phase of the question that attention has been directed within recent years, and to obtain evidence bearing upon it that the examination of gastric contents and feces for tubercle bacilli has again been resorted to by several groups of investigators.

Among the more recent reports is that of Gourley<sup>2</sup> who studied 59 cases by means of gastric lavage and animal inoculation. In 9 cases there were no demonstrable roentgenographic lesions, 50 presented such evidence, and all gave a positive tuberculin test.

Tubercle bacilli were present in the gastric contents of 28 or 47.4 per cent of the whole series and in 28 or 56 per cent of the 50 having demonstrable lesions.

That calcification of the parenchyma of the lung or regional lymph nodes is not indisputable evidence of entire arrest and inactivity was suggested by the fact of 12 children showing evidence of calcification in the film; 5 had tubercle bacilli in the gastric contents. Gourley concludes from her study that all types of parenchymal lesions in childhood tuberculosis, including McPhedron's "C" group, are open cases.

A series of 60 cases examined by Mishulow, Kereszturi, and Hauptman<sup>3</sup> gave 10 per cent positive findings in children apparently well when examined, 54.5 per cent positive findings in those slightly ill, and 63.1 per cent positive findings in children who were very ill. Of those with nondestructive parenchymatous lesions 24 per cent were positive while 83.3 per cent were positive in the group showing destructive parenchymatous pulmonary lesions.

Negative findings were encountered in all cases with only positive intracutaneous tests, hilum lymph node tuberculosis, pleurisy with effusion and tuberculous peritonitis.

In this series tubercle bacilli were found in the feces in 12 cases, but in variable numbers, in one case so few that they were only detected when the smears were reexamined after guinea pigs proved to be positive.

Although examination of the feces can be done repeatedly without discomfort to the patient the results are not quite as good as those from examination of the gastric lavage, and the procedure is, moreover, somewhat more complicated.

In the interpretation of such findings possible sources of error must, of course, be remembered and taken into account if they cannot be eliminated.

Thus, tubercle bacilli may be ingested with raw milk, tuberculosis of the stomach may exist but is so rare as to be of negligible importance, tuberculosis of the tonsil is also a somewhat rare possibility, and there may be tuberculous foci in the sinus or middle ear.

In considering the significance to be attached to the demonstration of tubercle bacilli in the gastrointestinal tract in children Nalbant<sup>4</sup> points out that there are various possible sources, among which are:

A tuberculous infiltration in the lung, such lesions usually being demonstrable clinically or by x-ray.

A receding type of childhood tuberculosis in which the lesions may have cleared roentgenographically though not pathologically.

A caseating tracheobronchial lymph node which may have broken into the trachea or a bronchiole or even through the esophagus.

Other possibilities are, as has already been noted, the presence of an upper respiratory tract tuberculous infection which, however, is uncommon; tuberculous enteritis or gastritis, quite uncommon, and the presence of a tuberculous growth in a bronchus or bronchiole. It has also been suggested by some, Mikulowski,<sup>5</sup> for example, that the source of tubercle bacilli in the feces may be infected bile which has regurgitated into the stomach. This, of course, could only be accounted for either by assuming a primary gallbladder lesion, certainly an unusual occurrence, or by the assumption that the infection of bile was the aftermath of a tuberculous bacteremia. In the latter case the complete escape from tuberculous meningitis or a generalized tuberculosis would be difficult to explain.

On the other hand, it is fair to assume that many, if not the great majority, of cases of tuberculous pleurisy, tuberculous bone and joint disease, or Pott's disease may well be localizations of a former bacillemia.

The problem is one well worthy of further study, not only from the standpoint of furnishing an additional avenue for the laboratory diagnosis of tuberculosis in childhood, but also, because of the clinical and social significance of the results obtained, which may only be evaluated after extensive study.

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—R. A. K.

#### Staff Meetings and Statistics

ONE of the requirements for an "approved" hospital is that there shall be a monthly Staff meeting at which the work done and the results secured by various departments shall be discussed and evaluated.

The principle underlying this provision is, without doubt, wise and well founded but if the reports thus made obligatory are to be productive of the results desired they must be something more than mere statistical recitals, and the discussion which they are intended to provoke must comprise something more than an exchange of compliments.

As every one knows, the value of statistics depends, not merely upon their volume, but upon the data from which they are compiled, the honesty with

which they are gathered, and the sincerity and care with which they are interpreted.

For the intelligent interpretation of statistical data one must take into consideration, not only the figures themselves, but also the many and varied aspects of the cases to which they refer. For it is obvious that during certain periods, for example, the admissions to a hospital may include many patients whose condition makes recovery problematical, many patients who, despite the fact that they may be justly regarded as unavoidably bad risks, must nevertheless be subjected to operation with an effect which may be foretold with some confidence upon the mortality statistics of the surgical service in question.

Nevertheless, the not uncommon practice of briefly and sometimes even cursorily reciting the number of cases of this, that, and the other thing together with a bald statement that so many were discharged "cured" or "improved," while it may conform to the letter of the law, falls far short of real compliance with the end in view.

The real purpose, it may be suggested with some justice, is to determine, not merely what happened, but why did it happen and, what is still more important, could it have been avoided?

Although speaking of operative mortality only, Lahey\* has enunciated principles which are equally applicable to medical services as well and might well be widely disseminated.

"While there will be certain years," he says, "in which many more serious cases will require operation than in others, and there will be others in which unavoidably bad risks must be accepted, nevertheless, the yardstick whereby one may measure surgical skill, surgical judgment, and the protection with which the patient is surrounded, is that of mortality."

This, of course, partakes somewhat of the nature of a truism but Lahey adds these significant qualifications:

"Mortalities are in some measure related to the surgeon's state of mind. If a surgeon assumes the position that a certain number of patients must die anyway following surgical procedures since, to be given a real chance of cure, particularly in carcinoma, radical procedures must be done, then it naturally follows that a certain number of mortalities will occur. . . . If one is prepared to expect mortalities, he has in some measure compromised his conscience to their occurrence. In my own opinion, the attitude should be that patients should not die following surgical procedures, that when they do die following surgical procedures, one's attitude, with reasonable exception, should be that an error in judgment has been made, either in advising operation at all, in the extent of the operation, in some of the technical procedures, or in the way of pre- or post-operative management. . . .

"It is extremely easy in surgery, particularly when dealing with patients who are in a desperate condition, to justify in one's mind desperate risks, but very rarely, in my experience, do desperate risks accomplish very much which is worth while. . . . When one knows and frankly faces the mortality rate this has the advantage of making one acutely aware of his errors in judgment and

\*Lahey, F. H.: Operative and Mortality Statistics of The Lahey Clinic for 1932, Bull. Lahey Clinic, 1932.

stimulating every one who is connected with operative procedure to his utmost efforts, pre- and postoperatively, to prevent the occurrence of a mortality."

These quotations present and embody an attitude equally applicable in principle to medical mortality statistics as well as surgical and deserve general consideration.

The development of such an attitude would without doubt remove from many Staff meetings the cut and dried and stultifying acceptance of mortality statistics with resigned equanimity and lead, on the contrary, to the awakening, not of acrimonious criticism, but of a spirit of earnest inquiry. To repeat what has already been said above, it is not the recital of what happened that is important and profitable but why did it happen and, more particularly, could it have been avoided?

It is hardly conceivable that there exists a physician or a hospital staff with the temerity to assert or the credulity to believe that, individually or collectively, they have never been guilty of an error of judgment, a failure to reach the proper diagnosis, an omission of something which might have been useful in the study of a particular case. How many can honestly deny an excessive and ill-warranted use, for example, of laboratory methods neither suggested nor indicated by the clinical data, the place of which they are all too often made to take?

How many times must honesty compel us to sigh with the unknown who regretted that his "foresight was not as good as his hindsight"?

And how many times are Staff meetings characterized by any such frank admissions or discussions?

It is particularly in the hospital of small or moderate size that such discussions would be most productive of benefit to all concerned, as it is also particularly in such hospitals that they are most often neglected.

The full function and entire duty of a hospital cannot be achieved by routine work, nor can it be expressed solely by the accumulation and recital of statistics relating to admissions, operations, and discharges.

Unless the data so accumulated are so studied and analyzed—critically, if need be, that continual improvement in methods and ability results, if not achieved is at least sought for, then Staff meetings, though complying with the letter of the law, are more apt to be boring and stultifying than inspiring or profitable.

Mistakes are, in the ordinary course of human events, more or less inevitable. It is not the mistake but the failure to learn from it which is disgraceful and leads to its repetition.

It is from our failures that we should learn rather than from our successes. And it is upon the discussion and analysis of our failures that we should spend our time leaving our successes to speak for themselves.

—R. A. K.

# *The Journal of Laboratory and Clinical Medicine*

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VOL. 20

DECEMBER, 1934

No. 3

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## CLINICAL AND EXPERIMENTAL

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### EFFECTS OF HYPERPYREXIA INDUCED BY PHYSICAL MEANS UPON COMPLEMENT-FIXING ANTIBODIES\*

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L. G. HADJOPOULOS, M.D., AND WILLIAM BIERMAN, M.D., NEW YORK, N. Y.

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THE hyperpyrexia occurring in acute infectious diseases has been considered as playing an important rôle in the production of immune reactions. This idea, however, has not been based upon exact experimental evidence because of the impossibility of separating the antigen factor (bacterial protein) from the consideration of the temperature elevation per se.

Of late years several methods have been developed for the elevation of systemic temperature by physical means. These include hot baths, diathermy, radiant heat (phototherapy), and radiotherapy (30-meter short wave electromagnetic radiation). We have elevated the systemic temperature in human and in animal subjects by means of radiotherapy and then maintained it by means of phototherapy. This has given us the opportunity of observing the serologic changes produced by hyperpyrexia uncomplicated by the presence of any extraneous foreign proteins.

The effect of hyperpyrexia on the nonspecific alexin (the natural complement of the blood) and on specific agglutinins (antityphoid) have been sufficiently studied to warrant the drawing of temporary conclusions. It has been shown that the alexin in the serum is lowered following exposure to radiotherapy. Similarly, the specific agglutinins induced by the parenteral introduction of typhoid vaccines in rabbits is also reduced, and by repeated exposures this specific titer can be completely lost.<sup>1</sup>

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\*From the Departments of Bacteriology and Physical Therapy of the Beth Israel Hospital. Received for publication, December 15, 1933.

In our experience, limited to a small number of experiments on rabbits exposed to radiothermy, we observed a definite, though slight, primary stimulation of the complementary titer of the serum followed by a fall to normal and even to subnormal figures. These findings were in conformity with our views on the production and disposal of complement as previously described with reference to malarial fever and to other infections.<sup>2</sup> The immediate effect of radiothermy on typhoid agglutinins was not as conclusive as that on the complement. Although a slight stimulation phase could at times be observed, the after-effects were invariably of the depressing nature, lasting from two to four days. The cumulative depressing effect of repeated exposures, as noted by us, are in accord with observations of other investigators.

As we know little or nothing of the effects of hyperpyrexia on the higher order of immune reactions, such as the production of complement-fixing anti-

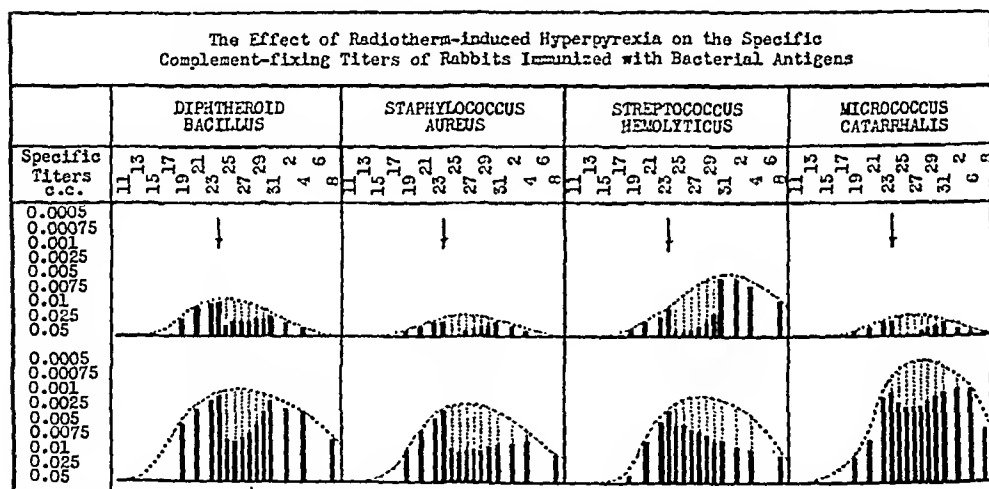


Chart 1.—The graph is drawn on the basis of data given in Table I. The heavy vertical lines stand for the daily titers of the specific complement-fixing antibodies. On the date designated by the arrow (July 24), animals of Series 1 and 3 were exposed to radiation. The course of immunity thereafter, instead of following the hypothetical curve (based on the immunity titers of nonradiated rabbits of Series 2 and 4) was reduced considerably and ran an irregular course. The ground thus lost is marked by the dotted lines.

bodies, we undertook to study this phenomenon in greater detail. Out of a healthy stock of 20 rabbits we kept four as controls. The remaining 16 were divided into 4 groups of 4 each. Each group was then immunized by repeated intravenous injections of one type of bacterial antigen (phenol killed bacteria in saline suspension) and the immunity response was watched closely by means of daily tests beginning on the fifth day following the last administration of antigen.

The bacterial antigens used in these experiments were of the common pyogenic microorganisms; staphylococcus, streptococcus, *micrococcus catarrhalis* and *Diphtheroid bacilli*. The daily titers of complement-fixing antibodies were determined quantitatively in terms of the least amount of serum capable of fixing a unit of complement in the presence of a unit of antigen.



In the course of these experiments we noticed that the stimulation of antibody production in rabbits was not uniform. Identical dosages of the same antigen stimulated some animals to a moderate degree and others to a markedly higher degree. We attributed this peculiar behavior in part to the variations in individual sensitivity inherent in animals in general. Some of the control rabbits showed a natural though very low-grade immunity to bacterial proteins. As the possibility of a latent focus of infection could not be excluded we were inclined to believe that such or similar factors were at

TABLE I\*

THE EFFECT OF RADIOTHERM-INDUCED HYPERPYREXIA ON THE SPECIFIC COMPLEMENT-FIXING TITERS OF RABBITS IMMUNIZED WITH BACTERIAL ANTIGENS

SERIES 1 AND 2	<i>Diphtheroid Bacillus</i>		<i>Staphylococcus Aureus</i>		<i>Streptococcus Hemolyticus</i>		<i>Micrococcus Catarrhalis</i>	
	R		R		R		R	
	1	2	1	2	1	2	1	2
Jul. 11-14	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.	c.c.
19	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
21	0.02	0.02	0.04	0.03	0.04	0.04	0.04	0.04
23	0.01	0.015	0.03	0.025	0.025	0.03	0.03	0.03
24	0.009	0.01	0.025	0.02	0.02	0.025	0.025	0.025
25	0.008	0.009	0.025	0.015	0.015	0.025	0.025	0.025
26	0.025		0.05		0.04		0.05	
27	0.03	0.009	0.05	0.015	0.045	0.02	0.05	0.025
28	0.025	0.008	0.05	0.01	0.05	0.015	0.05	0.025
29	0.025	0.008	0.04	0.01	0.045	0.01	0.05	0.03
30	0.025	0.009	0.035	0.01	0.03	0.009	0.045	0.035
31	0.02	0.01	0.03	0.01	0.025	0.008	0.035	0.04
Aug. 2	0.02	0.02	0.03	0.015	0.015	0.0075	0.03	0.045
4	0.025	0.025	0.025	0.02	0.005	0.0075	0.025	0.05
8	0.035	0.04	0.04	0.04	0.005	0.009	0.035	0.05
Reduction	0.05	0.05	0.05	0.05	0.008	0.025	0.04	0.05
Reduction	$\frac{1}{3}$		$\frac{1}{2}$		$\frac{1}{3}$		$\frac{1}{2}$	
SERIES 3 AND 4	R		R		R		R	
	3		3		3		3	
	3	4	3	4	3	4	3	4
Jul. 11-14	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
19	0.005	0.02	0.009	0.04	0.03	0.04	0.015	0.02
21	0.0025	0.0075	0.006	0.025	0.0075	0.03	0.0075	0.01
23	0.0015	0.005	0.003	0.02	0.004	0.025	0.001	0.007
24	0.001	0.0025	0.0025	0.015	0.0025	0.015	0.0009	0.005
25	0.0075		0.008		0.005		0.0015	
26	0.0075	0.002	0.0085	0.015	0.005	0.01	0.0015	0.003
27	0.0075	0.0015	0.009	0.015	0.005	0.009	0.002	0.0025
28	0.007	0.0015	0.009	0.015	0.006	0.008	0.002	0.002
29	0.0065	0.002	0.009	0.02	0.0065	0.0075	0.002	0.0015
30	0.005	0.003	0.009	0.02	0.007	0.0065	0.0015	0.001
31	0.0025	0.005	0.0085	0.025	0.0075	0.006	0.001	0.001
Aug. 2	0.0015	0.0065	0.0085	0.025	0.0075	0.0055	0.0009	0.001
4	0.002	0.01	0.008	0.03	0.008	0.005	0.0008	0.001
8	0.0025	0.03	0.0075	0.04	0.0085	0.005	0.0008	0.0015
Reduction	0.0075	0.04	0.01	0.05	0.01	0.0075	0.0004	0.002
Reduction	$\frac{1}{25}$		$\frac{1}{3}$		$\frac{1}{2}$		$\frac{1}{2}$	

\*R as appears in Series 1 and 3 indicates that these animals have been exposed to the radiotherapy treatment.

On dates July 11-14 rabbits received the antigenic injections.

On July 24, the first reading indicates the specific titer before exposure to radiotherapy, and the second immediately after the completion of treatment.

The specific titers of rabbits of Series 2 and 4 are also given in the table for the sake of comparing the course of immunity production with and without the effect of radiotherapy.

In the lines headed Reduction the figures indicate that the specific titers, after radiotherm exposure, have been reduced to these fractions of the original titers before exposure.

the basis of these variations. To minimize the influence of these variable factors we regrouped our animals according to the degree of immunity in them so as to include those with a moderate antibody production in Series 1 and 2, and the remainder showing marked antibody formation, in Series 3 and 4.

To test the effect of hyperpyrexia on the course of complement-fixing antibody formation, rabbits of Series 1 and 3 were exposed to radiotherapy for a sufficient length of time to raise their temperatures to  $41-42^{\circ}$  C. After these temperatures were attained they were maintained for three to four hours by exposing the animals to carbon filament electric lamps. The animals were bled immediately before and at the completion of the experiments and thereafter daily to observe the whole immunity phase. Animals of Series 2 and 4 were kept as controls to Series 1 and 3 so as to permit the determination of the immunity curves with and without radiotherapy. The results of these experiments are given in tabular and graphic forms (Table I and Chart 1).

The reduction in the complement-fixing titer of the serum after exposure to hyperpyrexia is due, in all probability, to a slowing up of the production of antibody rather than to its destruction. By observing the daily fluctuations in the antibody titers of treated rabbits we could note a delayed stimulation phase in the majority of these animals.

#### CONCLUSIONS

The production of hyperpyrexia by physical means causes a temporary diminution of the complement-fixing antibody titer of rabbits immunized against staphylococci, streptococci, *Micrococci catarrhalis* and *Diphtheroid bacilli*.

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## A CASE OF MERCURIC CHLORIDE POISONING TREATED BY EXSANGUINATION-TRANSFUSION\*

EDWARD H. HASHINGER, M.D., AND JOHN F. SIMON, M.D., LAWRENCE, KAN.

### HISTORY

ALTHOUGH the Egyptians as early as 1600 B.C. were acquainted with the use of mercury, they did not use it as a medicine, and Aristotle (384-322 B.C.) alone of the early Greeks prescribed it for certain skin conditions, as it was generally regarded too poisonous for internal use. However, it was used in India as a medicine possibly as early as 500 B.C. and old records show that mercurial gingivitis was known there as an entity. From India the knowledge of the medicinal value of mercury spread to Persia, and thence to Arabia; and after the birth of Christ it came into high repute as a universal panacea. Mesuë the Older (857) first carefully described mercury inunctions for skin disorders. About this time Arabian culture was rising above the European, and Avicenna and Constantinus Africanus described the toxic effects of mercury as pasty sores in the throat, bad breath, cramps, blood in the stools, disturbances of hearing and vision, and other of the familiar symptoms.

With the mingling of Arabian and European science through the Crusades, the method of mercury inunction gained root, even in the stronghold of Hippocratic teaching at Salerno, and during the Middle Ages came the development of the "salivation cures." Guy de Chauliac recognized the connection between mercury intoxication and salivation, but regarded it as a sign that the treatment was effective, since withdrawal of phlegm was considered as highly beneficial in gout. The Europeans found that mercury given either per os or by inunctions could cause diarrhea, thirst, anuria, and loss of appetite. Disturbances of vision, hearing, and speech were also noted.

Theories and speculations as to the action of mercury within the body were numerous and Thomas Sydenham (1624-1689) declared without doubt that mercury cured syphilis through salivation and that two to three liters of saliva was the proper amount for the patient to secrete each day. This type of treatment gave plenty of opportunity for the observation of mercury poisoning, and Jean Astruc of Montpellier divided the therapeutic or salivation stage from the intoxication stage; the latter, from his description of bloody stools, swelling of the face and bloody sputum, must have been near death from overtreatment.

John Hunter (1728-1793) declared that the toxic effects of mercury combined with the degenerative power of syphilis to act in double force upon the body, and this idea became enlarged upon until by the beginning of the nineteenth century, the symptoms of mercury poisoning and syphilis were

\*From the Department of Internal Medicine, University of Kansas.  
Received for publication, March 8, 1931.

much confused. A book was published in 1839 by J. P. Murphy showing how mercury alone caused the symptoms usually described as secondary syphilis.

It remained for Robert Overbeek in 1861 to show by clinical and experimental study the true nature of mercury poisoning. In the same year Kussmaul published a report showing the difference between chronic hydrargyrisms and constitutional syphilis. These two articles were the chief points in dispelling the many theories and confusion about mercury poisoning.<sup>1</sup>

#### TREATMENT

The last fifteen years have seen the treatment of mercury poisoning, particularly mercuric chloride, advance from questionable therapeutic measures prescribed by rote and usually without hope for the patient, to a sane, rational procedure, arrived at by experiment and observation, with a gratifying decrease in mortality. Before 1910, mercuric chloride poisoning was considered to be almost universally fatal, but Weiss<sup>2</sup> in 1924 reported 135 cases treated by the newer methods with but 8 deaths. Other men have reported equally good results, so it is apparent that considerable advance has been made in treating these cases.

The former conception has been that albuminous materials when combined with mercuric chloride rendered the poison insoluble and nontoxic; but this is not the case, for mercury albuminate is equally as toxic as the chloride. In fact any ingested mercury is first converted into the albuminate before it is absorbed, the difference being that it is not corrosive as is the mercuric chloride. Accordingly, it is to be remembered that the administration of albumin is to be followed by as prompt an evacuation of the stomach contents as possible.

The next step in treatment was to attempt to neutralize the absorbed mercury. Calcium sulphide was given enthusiastic acclaim in 1917 by Wilms<sup>10</sup> who found it gave excellent results if a freshly prepared solution was given intravenously within twenty-four hours after ingestion of poison. Ravaut<sup>3</sup> in 1920 described the use of sodium thiosulphate in treating arsenical dermatitis, and McBride and Dennie<sup>4</sup> popularized the use of this drug in converting toxic metals into nontoxic sulphides. Although Melville and Bruger<sup>5</sup> concluded from animal experimentation that sodium thiosulphate did not combine with mercury within the body, it remains the opinion of many men that it is of much benefit in treating mercuric chloride poisoning. Marchbanks, Church, and Smith<sup>6</sup> reported a case in 1931 in which the entire credit for saving the patient's life was ascribed to sodium thiosulphate given intravenously.

The Lambert routine of treatment aiming at elimination, alkalinization, and diuresis, has met with considerable favor and while not as popular as ten years ago, still has a number of valuable points. Modifications of this treatment were reported by Weiss<sup>7</sup> in 1917 and Rosenbloom<sup>8</sup> in 1919 with excellent results. Lemierre, Laudat, and La Porte<sup>9</sup> believed that sodium chloride in isotonic and hypertonic solution intravenously resulted in amelioration of symptoms and favored renal excretion. They reported two cases.

Surgery has been used in treating these cases for many years. Anderson<sup>11</sup> tried irrigating the colon through a cecostomy opening. He reported a case with recovery in which the operation was done fourteen hours after ingestion of the poison. Decapsulation of the kidneys has also been done but no longer holds much favor. More recently Berger and others<sup>12</sup> reported a series of cases in which it was shown that immediate cecostomy and constant colonic lavage was the best method of preventing and treating the gangrenous colitis of the mercuric chloride poisoning.

#### REPORT OF CASE

The case here reported is different from others in that the chief mainstay in the method of treatment is one to which we are unable to find any reference in the literature as having been used in human beings, namely exsanguination-transfusion. It was done for two chief reasons: first, as shown by Rosenbloom<sup>13</sup> and others, the blood and other tissue juices contain the greater part of the absorbed mercury, so by replacing the blood the poison could be removed in the largest possible quantity. Second, by replacing the blood in sufficient quantity, the nitrogenous products retained in the body would be diluted and kept at a sublethal level until the renal epithelium might regenerate. The same method has been of service in delaying uremic death in cases of chronic nephritis.

In 1915, Robertson<sup>14</sup> proposed exsanguination followed by transfusion in cases of severe bacterial toxemia and believed that it might be of value in treating chemical poisoning as well. Haskell and others<sup>15</sup> working with dogs (six in all) and using the minimum lethal dose of mercuric chloride as found by Sansum<sup>16</sup> bled the animals immediately after intravenous administration of the poison and transfused with matched blood. In spite of repeating the procedure, all the dogs died within an average time of twenty-four hours and the authors were of the opinion that the exsanguination-transfusion method of treating mercuric chloride poisoning was of no value. In 1917, Burmeister<sup>17</sup> showed by the same method that although the animals died, the degeneration of the kidney epithelium in dogs and rabbits was distinctly inhibited by the use of transfusions.

In our case in addition to the bleeding followed by transfusions, the patient was given a small amount of sodium thiosulphate for its reported value in neutralizing available mercury. Sodium chloride was given to combat loss of chlorides by vomiting.

M. C., white male, aged twenty-three, entered Bell Memorial Hospital May 22, 1933, with a chief complaint of inability to get his breath. Eight days previously in the evening he had swallowed 18 gr. (1.3 gm.) of mercuric chloride with suicidal intent. It was nearly an hour later before he vomited. He continued to vomit and choke for the remainder of the night. He was not seen by a physician until the following morning. The treatment for the eight days previous to hospital admission consisted of fluids, principally milk, and daily intravenous injections of sodium thiosulphate. He said that on the

third day he began getting short of breath but did not notice scanty urine until the following day. Bloody diarrhea was severe before admission to the hospital.

On admission he was gasping for breath, and his skin was bluish, dry, and cold. His breath was fetid. Blood oozed from the soft, swollen, inflamed gums, and there were hemorrhagic areas on the uvula. Moist râles were heard in the base of each lung. Examination of the heart revealed a rate of 110, blood pressure, systolic 170, diastolic 100, and there was redupli-

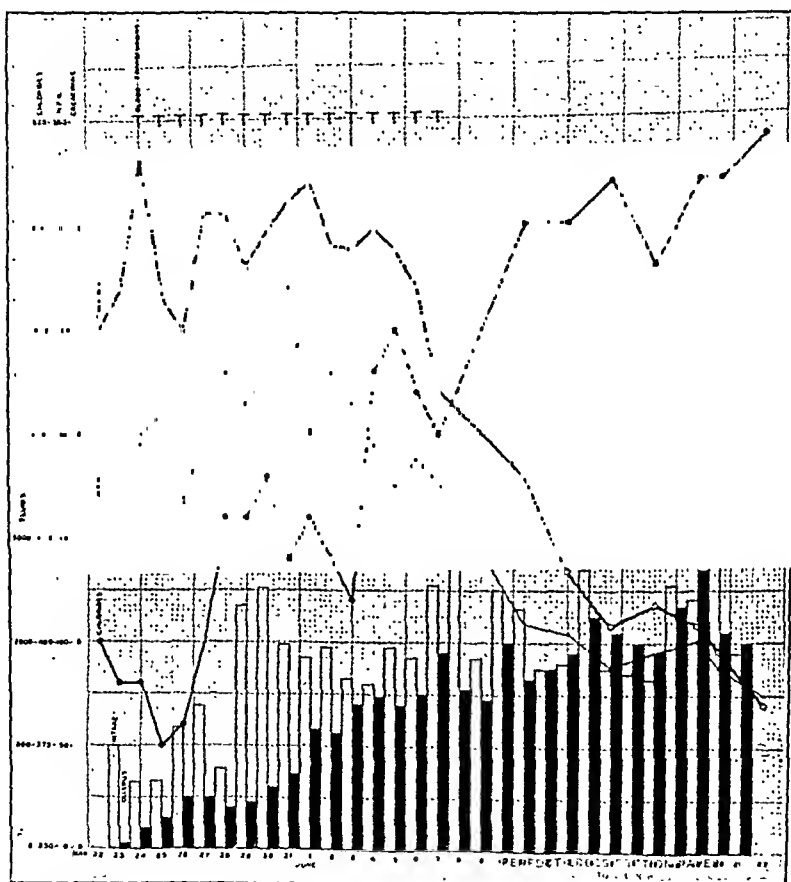


Fig. 1.

cation of the second mitral sound. The abdomen was distended and tender. The liver was palpated 10 cm. below the costal margin and its edge was rounded and tender. On admission the blood chemistry showed per 100 c.e.: sugar 83 mg., nonprotein nitrogen 167 mg., creatinine 12.5 mg., chlorides 400 mg. Urine showed a specific gravity of 1.014, strongly alkaline, trace of sugar, albumin two-plus, many hyaline and granular casts. Hemoglobin was 70 per cent, R.B.C. 3,160,000, W.B.C. 20,500, with 89 per cent polymorph-nuclears.

He was given fluids by mouth, 50 c.c. of 50 per cent glucose intravenously, and 15 gr. of sodium thiosulphate intravenously in the first twenty-four hours.

The patient seemed in about the same condition at the beginning of his second hospital day, but toward the end of this day he had more respiratory difficulty, developed auricular fibrillation, the cyanosis increased, and the end seemed to be near at hand. At this time it was decided to bleed and transfuse the patient. A list of 25 suitable donors was compiled and in the evening of May 24 (forty-eight hours after admission), 850 c.c. of blood was withdrawn and 1,000 c.c. of citrated blood given by indirect method. For the next two weeks the patient was bled an average of 700 c.c. and transfused 800 c.c. daily. On the third and fourth hospital days the patient seemed to be getting worse. His pulse became weak, the abdomen distended, and blood flowed freely from the gums. He rallied somewhat at the end of the fourth day and showed improvement, gradual at first, then more rapid as the urinary output began to increase. Fifteen grains of sodium thiosulphate were given intravenously daily for the first three days of hospitalization. Sodium chloride in 45 gr. dosages was given daily for the first four days, when vomiting was severe. There was no profuse diarrhea during the time he was in the hospital, but blood was recovered from the stools. After 15 daily transfusions were given they were discontinued because of clinical improvement in patient and the gradual increase in urine output.

At the time of dismissal, the gums were healed; the heart was regular and pulse normal; there was no dyspnea on exertion; no occult blood in the stools and the urinary output was normal. The blood chemistry records and fluid intake and urine output during the stay in the hospital are shown in Fig. 1.

The patient was seen seven months after dismissal (Jan. 22, 1934), when examination showed urine entirely negative. Blood pressure, systolic 140, diastolic, 80. Blood chemistry: nonprotein nitrogen 35.3, creatinine 1.6, urea 11.81, sugar 82, and chlorides 460. Clinically he was a well man.

#### CONCLUSIONS

1. Severe mercury poisoning is treated successfully by a new method, that of exsanguination and blood transfusion.
2. Sodium thiosulphate intravenously had seemingly proved unsuccessful in the treatment of this case.

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## NODULAR GRANULOMATOUS LESIONS OF THE LIVER, SPLEEN, AND LYMPH NODES

PROBABLY AN ATYPICAL FORM OF HODGKIN'S DISEASE\*

DONALD C. BEAVER, M.D., AND ALBERT M. SNELL, M.D., ROCHESTER, MINN.

**I**N A recent paper, Beaver and Johnson<sup>1</sup> have described the case of a young woman who died following a typhoid-like illness associated with fever, marked abdominal distention, diarrhea, and terminal coma. The striking nodular, necrotic lesions in the liver and spleen of this patient were at first thought to represent a form of tularemia, but further study convinced the writers that they were dealing with an unusual type of Hodgkin's granuloma. Since no corresponding example of this disorder has to our knowledge been reported in recent literature, it seems desirable to report a second case in which the terminal clinical picture resembled that of portal cirrhosis, and in which similar nodular granulomatous lesions of the liver, spleen, and lymph nodes were found at necropsy.

While neither the first case nor that presented in this report can as yet be accurately classified on either a clinical or pathologic basis, they are reported in the hope that other observers may be able to correlate them with similar cases and thus clarify their position in respect to other and better recognized granulomatous processes.

### REPORT OF A CASE

A housewife, aged twenty-seven years, first registered at The Mayo Clinic in July, 1924, complaining of swelling of the left side of the neck. The past history was irrelevant, except for many attacks of tonsillitis in early life. Five years before, tonsillectomy and removal

\*From the Mayo Clinic.

Submitted for publication March 20, 1934.



of the lymph nodes of the left side of the neck had been performed elsewhere. Details as to the histologic appearance of these nodes were not available, but the patient assured us that they had been regarded as tuberculous.

On physical examination the patient was in good general condition except for a mass of firm, discrete lymph nodes in the left cervical region. A single enlarged node was also noted in the right axilla. These nodes varied from 0.5 to 4 cm. in diameter, the largest being situated directly behind the angle of the jaw. Laboratory examinations including roentgenograms of the thorax, urinalysis, blood count, and Wassermann test of the blood, gave negative results. The patient did not wish to have a node excised for biopsy and, consequently, radium treatment was advised. A total of 3,000 mg. hours of radium was applied to the left submaxillary and upper and middle cervical areas, and 2,000 mg. hours to the right axilla. Two months later another course of radium treatment was given.

The patient's second admission to the clinic was in November, 1925. She stated that the nodes previously described had been greatly reduced in size after treatment with radium, but that in the summer of 1925 they had become enlarged again and the node in the right axilla had caused her some pain. Results of physical and laboratory examinations were essentially the same as at the time of her previous examination. A third course of 5,000 mg. hours of radium was given over the involved nodes with satisfactory results.

The patient's third examination was in July, 1932. During the preceding seven years the nodes had alternately increased and decreased in size, but she had had no general symptoms until shortly before admission, when loss of weight, nausea, and vague indigestion had been noted. On physical examination the same mass of nodes that was present eight years previously was noted in the left cervical region, extending from the mastoid region to the clavicle. Physical examination was otherwise not remarkable except for definite enlargement of the liver which was palpable about 4 cm. below the right costal margin. The tip of the spleen could also be felt. A careful search was made for a distant tuberculous focus, but nothing definite could be demonstrated. Roentgenographic examination disclosed slight widening of the mediastinum, but otherwise laboratory examinations gave negative results. Because of the unusual persistence of this cervical adenopathy, the patient was again advised to have a node excised for biopsy. This she declined to have done and asked that another course of radiotherapy be given. Because of the questionable mediastinal shadow, the mediastinum was included in this course of treatment. As on previous occasions, radiotherapy caused progressive decrease in the size of the nodes, but she failed to gain in strength. About three months after the completion of treatment she had an attack of herpes zoster, limited to the left lower portion of the trunk, and following this she was almost disabled and was confined to her bed. At this time two new developments were noted: slight fever in the afternoon and progressive increase in the size of the abdomen. The latter was associated with pain in the right upper quadrant, which varied from vague discomfort to actual distress requiring codeine for relief. By December, 1932, a slight hacking cough with a small amount of blood-tinged sputum had developed, and there was also definite edema of the lower extremities.

The patient was readmitted to the clinic in January, 1933, as an emergency case. On physical examination the striking findings were marked pallor, an extreme degree of ascites, and moderate peripheral edema. The cervical nodes were much reduced in size and could be felt only as discrete, pea-sized masses. The thorax was essentially normal, except for an elevated diaphragm and evidence of congestion at the bases of both lungs. Pelvic and rectal examinations, while somewhat unsatisfactory because of the ascites, revealed no findings of any importance. Urinalyses were repeatedly negative, except for an occasional hyaline cast and faint traces of albumin. The value for hemoglobin was 10.5 gm. per 100 c.c. of blood, erythrocytes numbered 3,220,000 and leucocytes 3,400 per cubic millimeter of blood: The differential count was approximately normal, and the blood smears revealed active regeneration of erythrocytes and moderate polychromatophilia. The concentration of bilirubin in the serum was 2.9 mg. and the reaction direct. The value for urea was 18 mg. per 100 c.c. of the blood. Roentgenographic examination of the thorax and fluoroscopic examination of the stomach gave negative results. Electrocardiograms showed diphasic T-waves in derivations

1, 2, and 3, but were otherwise normal. A test of hepatic function with bromsulphalein revealed dye retention Graded 4. A tentative diagnosis of portal obstruction with ascites was made, the most likely cause being regarded as portal cirrhosis with secondary splenomegaly. Treatment with diuretics was begun, but the patient reacted so poorly that it was necessary to perform paracentesis on the sixth day of her stay in the hospital. At that time 6,000 c.c. of dark, amber-colored fluid were removed. Cultures were made, but no growth of organisms was obtained. Stained smears from the ascitic fluid were negative for *Mycobacterium tuberculosis* and other organisms. Following this paracentesis, no improvement was noted. The abdominal and pelvic organs were examined carefully after the removal of the ascitic fluid, but nothing could be made out other than the enlargement of the liver and spleen previously mentioned. Throughout the patient's stay in the hospital she had more or less continuous elevation of temperature, which sometimes reached 102° F. Much of the time she was somewhat drowsy and dull, but she was never definitely disoriented. Because of the probability of impending hepatic insufficiency, a liter of 10 per cent solution of glucose was given intravenously daily. In spite of all attempts to produce diuresis, the ascitic fluid slowly reaccumulated, and it was obvious that another paracentesis would soon have to be performed.

Because of the possibility of peritoneal tuberculosis, it was decided to explore the abdomen through a small incision and examine the liver, spleen, and peritoneal surfaces. This was done January 19 by H. K. Gray, the exploration being carried out through a right rectus incision. The omentum and peritoneal surface appeared entirely normal. The hepatic surface was irregular and roughened, suggesting an atrophic type of cirrhosis. The spleen was about six times the normal size and presented no unusual features. The pelvic organs were examined, but no evidence of pelvic tuberculosis could be demonstrated. Talma-Morison omentopexy was performed, and the abdomen closed in the usual manner. The patient failed rapidly after operation, lapsed into coma and died on the fourth postoperative day with signs of hepatic insufficiency.

Necropsy was performed three and a half hours after death. The body was unembalmed. The ankles were edematous, Graded 2. The recent surgical wound in the right upper portion of the abdomen was clean. The peritoneal cavity contained 3,000 c.c. of clear straw-colored fluid; otherwise the peritoneum was normal in appearance. The right pleural cavity was practically obliterated by fibrous adhesions and contained no excess fluid. In the left cavity there were no adhesions, but 150 c.c. of serosanguineous fluid was present. The lungs were essentially normal except for atelectasis of the lower lobes and the presence of calcified tubercles in the right middle and left lower lobes with fibrocaseous tuberculous involvement of the hilar lymph nodes. The pericardial sac contained 65 c.c. of clear straw-colored fluid. The heart was normal in appearance.

The spleen weighed 557 gm. Multiple dark red, grayish pink, or entirely gray nodules of increased consistency were distributed quite generally beneath the capsule and throughout the splenic pulp. These varied in size from 0.5 to 2.5 cm. in diameter and frequently were spherical or irregular in shape and fairly well circumscribed; often, they were slightly raised as rounded nodules when present beneath the capsule. The appearance of some was similar to that of infarction.

The liver weighed 2,533 gm. The consistency was somewhat decreased. The color was light reddish yellow with dark red or grayish patches mottling the surface. The capsule was smooth and glistening and not thickened. The dark red and gray areas rose above the surface slightly as rounded elevations, and measured 1 mm. to 2 cm. in irregular diameter. The appearance of the

sectioned surface was similar to that of the capsular portion. Throughout all parts of the liver the hemorrhagic areas were prominent. They were slightly firmer than the remainder of the liver and devoid of the lobular markings that were well revealed elsewhere. This peculiar mottling of the liver was quite dissimilar to that seen in chronic passive congestion, since the hemorrhagic zones were very irregular in size, shape, and in distribution, and appeared not to be confined to central lobular positions, but, on the contrary, seemed to involve entire lobules or groups of lobules.

Lymph nodes surrounding the tail and head of the pancreas, the porta hepatis, and in the right axilla, were enlarged, of slightly increased consistency, and glistening, grayish white to pink in color with irregularly mottled deep red zones similar in appearance to the nodules in the spleen. Lymph nodes elsewhere were somewhat enlarged and of slightly increased consistency, but, generally speaking, there was nothing especially remarkable about them.

The entire gastrointestinal tract, pancreas, kidneys, suprarenal glands, ureters, bladder, uterus and adnexa, thymus and thyroid glands were normal. The sclerosis of the aorta was Graded 1. The brain was not examined.

In microscopic preparations\* of the relatively normal portion of the liver, which constituted less than one-half of the hepatic bulk, there was moderately severe fatty change. Hepatic cells not so affected were usually faintly stained. Central vein areas were irregularly congested, with sometimes slight atrophy and necrosis of central hepatic cords. Throughout all portions of the liver there were cellular collections consisting of lymphocytes and a few endothelial cells. The cellular collections, when small, were confined to the periportal connective tissue, but when larger, they extended through the peripheral portions of the lobules or involved zones as large as, or larger than, a lobular unit. When the aggregations of cells were larger, they were accompanied by areas of necrosis (Fig. 1 A) of varying size, always surrounded by lymphocytes. There was no sign of rapid progression of the necrosis to surrounding hepatic parenchyma, for the hepatic cells near the periphery of these areas were as normal in appearance as those anywhere in the liver. The areas of necrosis were sometimes devoid of all cellular detail, but this varied, for in some, congested sinusoids and reticular cells persisted. In other places there was hyperplasia of reticulum. The cells which we have called "lymphocytes" were morphologically like mature lymphocytes, though perhaps slightly larger. The nuclei were large hyperchromatic structures, and the chromatin was often finely divided. There was a scant amount of pale-staining cytoplasm about some cells, although usually none could be identified. The cells described as endothelial were large, with large, pale nuclei and with abundant cytoplasm, sometimes pale and sometimes intensely acidophilic. A few of these cells were phagocytic for erythrocytes and cellular debris. Occasionally these cells had giant nuclei, and a few were multinucleated, the latter being usually hyperchromatic. The reticular cells were usually spindle-shaped or stellate, sometimes with fine fibrillae at the poles of the cells. Mitotic figures were not identified in any of these cells.

\*Tissues fixed in Orth's fluid, embedded in paraffin, and stained with hematoxylin and eosin.

Microscopic sections of the spleen, taken through the red nodular areas, disclosed intense sinusoidal congestion. There were relatively few lymphocytes in the pulp, and malpighian corpuseles were small. In areas of less intense congestion, or at the periphery of the fairly sharply circumscribed hemorrhagic areas, there were proliferated reticular cells and collections of lymphocytes. The lymphocytes were sometimes collected in small aggregations with the hyperplastic reticulum, but often they were independent. The reticular cells were arranged as bands or, in many places, small foci of cells. Sometimes there were small zones of necrosis, surrounded by lymphocytes. Many of the small malpighian follicles of these areas contained masses of hyaline-fibrinoid material. Sections of the white nodular areas revealed extensive necrosis, as in infarction;



Fig. 1.—A, Liver: two foci composed of lymphocytes and a few endothelial cells. In the lower lesion there is also proliferation of reticulum and necrosis. The upper lesion is small and confined to a periportal space. Extensive fatty changes exist in the hepatic cells (hematoxylin and eosin  $\times 90$ ). B, Spleen: A splenic trabeculum with foci of lymphocytes, also perivascular arrangement of lymphocytes (hematoxylin and eosin  $\times 90$ ).

only a few well-stained lymphocytes were present. At the periphery of these areas there was a certain amount of blending with surrounding splenic pulp. In the places of transition, there were hyperplastic reticular cells and foci of lymphocytes. The lymphocytic collections were particularly evident in the connective tissue of the trabeculae, in the walls of larger vessels and surrounding them (Fig. 1 B). In the dense connective tissue the lymphocytes tended toward columnar arrangement.

Lymph nodes of the splenic and hepatic hilum and in the head and tail of the pancreas usually disclosed complete loss of normal nodal architecture. In

the nodes which revealed only mild involvement there was only hyperplasia of the reticulum with some atrophy of lymph follicles. Lymph sinuses were often engorged with endothelial cells or obliterated by hyperplasia of the reticulum (Fig. 2 A). In nodes with extreme involvement neither lymph follicles nor sinuses could be identified and, in addition to areas of reticulo-endothelial cell hyperplasia and dense foci of lymphocytes, there were zones of necrosis apparently in the centers of dense foci of lymphocytes. There were also other large relatively acellular areas. These were well stained with eosin and by careful observation were seen to have a fibrillar or collagenous fibrous structure with few stellate or spindle nuclei and a few irregularly distributed lymphocytes (Fig. 2 B). At the periphery of these areas dense collections of lymphocytes

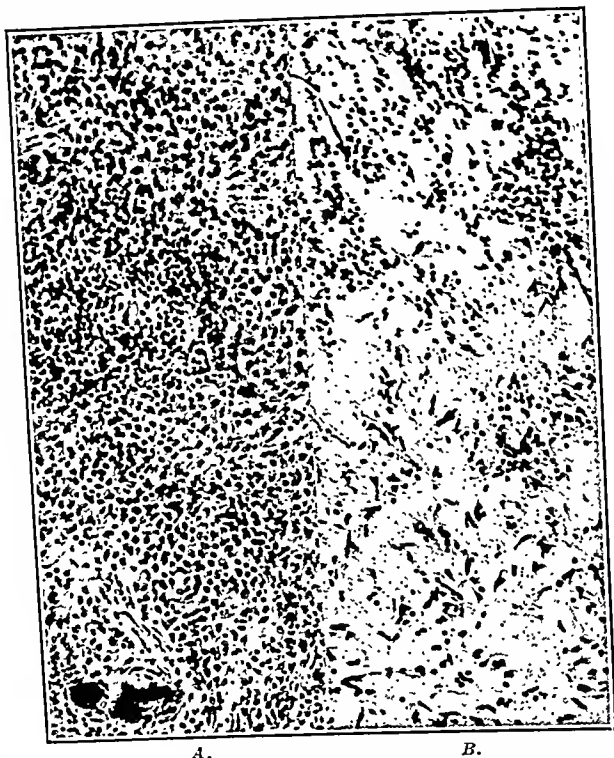


Fig. 2.—A, Mesenteric lymph node revealing loss of lymphoid follicles, engorgement of sinuses by large endothelial cells, and proliferation of reticulum (hematoxylin and eosin  $\times 150$ ). B, Pancreatic lymph node disclosing loss of lymphoid follicular architecture, proliferation of reticulum with fibrillar and collagenous connective tissue changes (hematoxylin and eosin  $\times 150$ ).

intermingled with a few larger endothelial cells were especially prominent. The capsules of the nodes were often densely infiltrated with lymphocytes and a few endothelial cells (Fig. 3 A). The lymphocytes and endothelial cells appeared to be mature and did not contain mitotic figures. Sections of mesenteric lymph nodes were similar to those of the pancreatic group that revealed the milder changes. There was focal hyperplasia of reticulum, and sinuses were choked with large endothelial cells. Capsules of the nodes were infiltrated with lymphocytes. Sections of cervical nodes revealed complete loss of follicle structure, reduction of lymphoid elements and sinuses, hyperplasia of reticulo-endothelial

cells, and early fibrosis. There were also many dilated congested blood vessels within the nodes. The capsules were thickened and contained much collagenous fibrous connective tissue. One peribronchial lymph node disclosed a caseous and calcified tubercle without apparent activity. In others there was loss of normal follicular structure. Sinuses were engorged with endothelial cells and lymphocytes and there were areas of marked proliferation of reticulum and fibrosis. Capsules were densely infiltrated with lymphocytes and a few endothelial cells. There were also areas of relatively acellular hyalin deposits containing but few spindle cells and lymphocytes. A lymph node from the right axilla disclosed complete loss of follicular and sinusoidal architecture and replacement by wide



Fig. 3.—A, Pancreatic lymph node and capsule; peripheral nodal lymph sinuses are entirely obliterated; capsule is diffusely infiltrated with lymphocytes and endothelial cells (hematoxylin and cosin  $\times 55$ ). B, Axillary lymph node; revealing wide bands of collagenous connective tissue in which a few lymphocytes and stellate and spindle-shaped cells may be seen. The entire node presented this appearance (hematoxylin and cosin  $\times 175$ ).

collagenous fibrils often arranged in wavy bands. With the hyalin there were spindle-shaped and stellate contracted nuclei and a few lymphocytes arranged in columns (Fig. 3 B). Lymphocytes were more numerous near the capsule, which was thickened and composed of dense fibrous connective tissue of collagenous type and contained a few collections of lymphocytes and endothelial cells. A lymph node from the lesser curvature of the stomach was identical in appearance with the node from the right axilla. The morphologic characteristics of the various cells described in the spleen and lymph nodes were identical with those previously described in the liver.

Sections of the lungs were normal except for a healed tubercle in the right middle and in the left lower lobes. The tubercles were fibrocaseous with calcification and without evident activity. In one of the tubercles there was deposition of bone and bone marrow formation.

The microscopic preparations of the left suprarenal gland revealed lymphocytic collections in the medulla. The capsule and surrounding areolar tissues were densely infiltrated with lymphocytes and a few larger endothelial cells. There were areas of necrosis and other places of marked fibrosis. In the areas of fibrosis the lymphocytes were often in dense masses or in columns. Lymphocytes were always mature in type, so far as could be determined. Mitotic figures were never found. Endothelial cells similarly were apparently mature.

Cultures of the blood, spleen, and liver were taken postmortem. They were prepared by inoculating material of the three types mentioned, on dextrose brain broth, and on hormone agar with sterile human blood added to the liver and spleen cultures. Incubation was carried on at 37° C. under aerobic and anaerobic conditions, over a period of three weeks. A few colonies of *Staphylococcus aureus* developed from each cubic centimeter of the patient's blood and from preparations of the liver and spleen. Three guinea pigs were inoculated intraperitoneally, and three subcutaneously with liberal amounts of the patient's blood and with triturated suspension of liver and spleen. Two rabbits were inoculated intravenously with triturated suspensions of liver and spleen. The animals were observed for a period of sixty days, temperatures of the guinea pigs being taken daily the first two weeks, and at no time did they reveal evidence of reaction. Finally all were killed and carefully examined. There were no lesions observable in any of them. Serum from the patient's blood was tested for agglutinins of *Brucella abortus*, *Pasteurella tularensis*, *Pfeifferella mallei* and *Proteus X19* with negative results. Sections of spleen, liver, and lymph nodes were stained by Brown-Gram stain and carbol-fuchsin acid-fast stain, without revealing anything except a few micrococci in certain lymph nodes.

#### COMMENT

The principal clinical features of this case were: first, the long-continued enlargement of the chain of cervical lymph nodes which had repeatedly diminished in size following radiotherapy, and second, the development of hepatic and splenic enlargement, followed by ascites, low-grade fever, and evidence of hepatic insufficiency as shown by clinical signs and also by tests of hepatic function. The latter findings hardly seemed compatible with the diagnosis of tuberculosis, which had previously been made on the basis of a biopsy of a cervical node. For this reason, the terminal phenomena were looked upon as independent of the previous adenopathy, an error which was apparent only at necropsy.

Just as the clinical features of this patient's illness presented complex problems in differential diagnosis, so also did the findings at necropsy. The only evidence of tuberculosis that could be found postmortem was in the form of primary healed foci in the lungs and peribronchial lymph nodes. The diagnosis of tuberculous cervical lymphadenitis was not confirmed by necropsy and, as a matter of interest, the cervical lymph nodes, so far as could be observed, were not remarkable in any respect. They were discrete, of the size of a pea or

smaller, and somewhat increased in consistency. Search was made in the axillary fossae for enlarged nodes because the history of the patient's illness indicated that involvement of lymph nodes in the right axilla had been present. Only one enlarged node, approximately 1 cm. in diameter, was found, this being very firm and grayish white. The clinical and roentgenologic evidence of widening of the mediastinal shadow was not confirmed. Aside from the one node involved by calcified tuberculosis, as already mentioned, there was nothing noteworthy grossly in this region. The really prominent lesions, those apparently responsible for the patient's demise, were confined to the abdominal cavity where there were found ascites, extensive lesions of the liver and spleen and of the lymph nodes composing the lymphatic chain across the abdomen between liver and spleen. The splenic and hepatic lesions grossly resembled multiple foci of neoplastic tissue, such as one might expect in certain types of sarcoma. Some of the lymph nodes were very large and formed masses along the pancreas which were composed of individually discrete nodes, conglomerate only because of loosely adherent intervening areolar tissue. Although not entirely typical, these nodes resembled grossly those seen in lymphosarcoma or Hodgkin's disease.

The microscopic preparations of the various lesions were even more difficult to evaluate accurately. From gross examination it had been anticipated that the lesions would be revealed microscopically as clearly defined Hodgkin's disease or sarcoma. But in contrast to this we found a rather indefinite granulomatous process with lymphocytic collections and extensive focal areas of necrosis in the liver and spleen. In the lymph nodes, there were foci of lymphocytes, hyperplasia of endothelial and reticular cells and zones of necrosis or fibrosis, with loss of sinus architecture and lymph follicular structure. The lesions in the cervical, peribronchial, axillary, splenic, pancreatic, hepatic, gastric, and mesenteric lymph nodes seemed to be of the same fundamental type, with differences only a matter of degree or age. In the liver and spleen, too, the lesions appeared to be pathogenetically related to those of the lymph nodes, representing more severe or earlier manifestations of the same process.

Such changes could hardly be interpreted as neoplastic in the usual sense. They were quite evidently an unusual manifestation of some unknown infectious agent that had produced in the tissues granulomatous reactions of nonspecific type, or at least of a type not to be identified with the granulomatous diseases of known etiology. Not only did morphologic study of the lesions eliminate from consideration the usual forms of specific granulomatous disease, but this had also been done by tissue stains for microorganisms, bacteriologic, serologic, and animal inoculation studies. Etiologic agents of the easily proved infectious diseases were thus eliminated, as well as tuberculosis (mammalian and avian), glanders, syphilis, tularemia, undulant fever, spirochetal diseases of the liver and the mycotic and protozoan diseases.

The presence of *Staphylococcus aureus* as found in the lesions postmortem could hardly be regarded as significant. They often have been found associated with the lesions in Hodgkin's disease; however, they are generally regarded as secondary invaders. In this connection Cunningham stated that: "Staphylococci and streptococci may give rise to a picture simulating Hodgkin's disease histologically, either by direct infection or as an irritative reaction." It had



been our opinion that the staphylococci in our case were secondary or terminal invaders and had in no way been connected with the evolution of the lesions, though no doubt final disposition of this question should be held in abeyance. Wallhauser considers, bacteriologically, that Hodgkin's disease presents "confusing and variegated findings, none of them generally accepted as etiologic."

It is apparent then, that in spite of ordinarily adequate study, the specific etiologic agent responsible for the lesions in our case had missed detection. Of the granulomatous diseases with which our case might be classified, that is, granulomas with unknown etiology, primary consideration must be given to Hodgkin's disease. In retrospect, the history of the case would coincide with such a diagnosis, and the findings, and at necropsy the morphologic studies, although not classical, could be considered sufficiently characteristic to admit this diagnosis. The possibility must be considered that in our case the unusual picture had been evolved by some modifying agent, such as might be produced by radium and secondary infection, also, that the disease existed in two phases, both of which are sometimes difficult to diagnose, the first being the latent or fibrotic stage and the second the earliest or acute phase. Cunningham stated that: "The earliest pictures of Hodgkin's disease are like those of any infection or irritation, a hyperplasia of lymphoid elements, and though the disease be suspected clinically, a histologic diagnosis cannot be made, although probable or suspected Hodgkin's is justly resorted to." Likewise of the end stages he stated: "The diagnosis cannot be made from such a gland or differentiated from the end-results of an acute lymphadenitis, tuberculosis, or syphilis."

Attention has been called to the case previously reported by Beaver and Johnson, in which the pathologic changes in the liver, spleen, and lymph nodes resembled very closely those found in the patient under discussion. In their patient, the clinical course of the disease was brief, but, in spite of this, sufficient portal obstruction had been produced to cause distention and ascites. Their search of the literature revealed two somewhat similar cases, that of Loygue and Clarion and that of Spangenberg. In each of these cases the general picture was that of a granulomatous process confined to the liver, spleen, and abdominal lymph nodes, and in each the histologic appearance of the tissues, while not typical, apparently resembled somewhat that of Hodgkin's disease.

There is a certain justification for temporarily including within the scope of the last named disorder other similar, although not identical, granulomatous diseases that are also of unknown etiology. At least it is on this premise that we must rely to support the diagnosis in this case. Others contend, perhaps more correctly, that so-called atypical cases of Hodgkin's disease should not be included in the same category, for such inclusion tends to impede discovery of etiologic factors. It must be admitted, however, that the criteria of histologic differentiation are not always well defined in this group of diseases and that there are multiple exceptions and overlappings, from varied granulomatous reactions to lymphosarcoma, leucemia, or pseudoleucemia. It is not entirely certain, moreover, that some of the conditions more recently described under the general heading "reticulosis" are not related to Hodgkin's disease. So long as indefinite definitions exist in such groups of diseases of unknown etiology, it will be extremely difficult to define sharply the scope of any one disease.

Ewing summed up this perplexing situation in his discussion of Hodgkin's disease with the following remarks: "Unfortunately, the clinical picture of Hodgkin's disease is not always associated with this particular histologic structure (proliferated endothelial cells, endothelial giant cells, plasma cells and eosinophilic leucocytes). . . Without the knowledge of the etiologic factor it remains almost as difficult as before to determine the true scope of this infectious granuloma, for all phases of the disease cannot be recognized with the same certainty. . . . In this dilemma one may follow the plan adopted by H. Ziegler, and include under Hodgkin's disease all conditions which seem to have any probable relation to the specific process. . . . There is a strong probability that the scope of the disease is very wide. . . ."

For these reasons and because of the clinical features of cervical adenopathy, which preceded the abdominal signs by a period of fourteen years, it seems advisable to record this unusual case as an example of atypical Hodgkin's disease. This is done with the full expectation that subsequent observations on similar cases may prove this classification to be in error. The possibility of a hitherto unrecognized disease entity, related to the infectious granulomatous processes, cannot be entirely excluded.

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## ALLERGIC PHASES OF ARTHRITIS\*

GRAFTON TYLER BROWN, B.S., M.D., F.A.C.P., WASHINGTON, D. C.

SERUM sickness manifests itself in about 90 per cent of the cases where any foreign serum has been administered intravenously, and, according to various estimates, in from 8 to 80 per cent where the serum is given subcutaneously or intraspinally. The symptoms of serum sickness appear in from eight to twelve or more days following the injection of the serum, and are thought to be due to an excess of antibodies, thrown off into the blood and tissues, coming in contact with some of the remaining serum proteins. Definite joint symptoms occur in about 14 per cent of all cases of serum disease. Therefore, in every case of acute arthritis or arthralgia, inquiry should be made as to whether any foreign serum has recently been administered. If the joint involvement is an anaphylactic manifestation of serum sickness, it is usually accompanied by other symptoms, namely, fever, enlarged glands, and skin rashes which are usually of an urticarial nature, but may be erythematous, morbilliform, or scarlatiniform in type. The ideal treatment for serum sickness with its accompanying arthritis is epinephrine hypodermically, followed by ephedrine orally.

The joint manifestations of acute rheumatic fever are, in all probability, due to bacterial allergy. In this disease the symptoms migrate from one joint to another within the space of a few hours, and it does not seem possible that any localized infection could change its site so rapidly. The only logical explanation is that the joint involvement is due to a toxic or allergic effect from a focus of infection elsewhere in the body, usually the tonsils. The presence of infection alone is not sufficient to account for the rheumatic symptoms, because in subacute bacterial endocarditis the blood is filled with streptococci, but there is no arthritis; whereas in rheumatic fever, positive blood cultures are obtained with difficulty, yet many joints are affected.

The striking periodicity of the attacks of intermittent hydrarthrosis, as pointed out by Miller,<sup>1</sup> and the satisfactory response of this condition to injections of peptone, strongly suggest an allergic basis. Rowe<sup>2</sup> reported a case of intermittent hydrarthrosis that was proved to be due to food sensitization.

Chronic rheumatoid or atrophic arthritis affects young adults and middle-aged persons, mostly women. This form of arthritis is undoubtedly infectious in origin, inasmuch as brilliant cures have been reported from removal of foci of infection during the first year of the disease. Although cultural studies in rheumatoid arthritis frequently reveal streptococci, these are not always of the same type, as evidenced by their different effect on the various sugars, and by the failure of cross agglutination. Nevertheless, in spite of the difference in the causative organisms, the pathology of the joint lesions is always the same, sug-

\*Received for publication, April 4, 1934.

Presented as part of a Symposium on Arthritis at a meeting of the George Washington University Medical Society, Washington, D. C., November 18, 1933.

gesting that the susceptibility or sensitization of the host is the important factor. A comparable situation exists in asthma and hay fever, where the same symptoms in different individuals may be due to sensitization to different and even unrelated substances.

Turnbull<sup>3</sup> stated that 60 per cent of a large series of arthritis patients, studied during a period of ten years, gave positive cutaneous reactions to tests with food allergens, and that elimination of the reacting foods from the diets of these patients resulted in a definite improvement in the arthritis. Rowe<sup>2</sup> also reports some cases of arthritis that are due to food sensitization, as evidenced by the prompt disappearance of the arthritis when certain foods were eliminated from the diet, and the recurrence of the trouble when the foods were again ingested. In Rowe's cases, however, skin tests were mostly negative and the causative foods were determined by means of his elimination diets.

The allergic hypothesis in arthritis obtains strong support from the experiments of Freiberg and Dorst,<sup>4</sup> in which they demonstrated that rabbits, after two or more injections of bacterial filtrates, developed characteristic joint lesions. These arthritic lesions became aggravated following each additional injection, and the rabbits gave positive skin reactions when tested intradermally with the bacterial filtrates.

Harkavy and Hebal<sup>5</sup> reported nine cases of asthma in adults who had also developed arthritis. In eight of these nine cases, the arthritis cleared up and the asthma greatly improved following the removal of the foci of infection. They concluded, therefore, that in these cases the arthritis, as well as the asthma, was due to bacterial allergy.

The possibility of food or bacterial allergy should be considered in every case of arthritis. A personal or family history of the common allergic diseases, namely, asthma, hay fever, urticaria, angioneurotic edema, eczema, or migraine headaches, greatly increases the likelihood that the arthritis also is due to some form of sensitization. Food sensitization should be ruled out by skin tests, both cutaneous and intradermal, with all the foods entering into the routine diet of the patient. If these food tests are negative, yet the history suggests food sensitization, then elimination diets are worthy of trial. Skin tests with stock polyvalent bacterial proteins should be done almost routinely in arthritis. Definite reactions with bacterial proteins are relatively rare, but when they do occur, treatment with corresponding vaccines, stock or autogenous, usually gives gratifying results. It must be remembered that although positive food tests usually give immediate urticarial reactions, bacterial reactions are almost always of the delayed inflammatory type.

In addition to the elimination of all foci of infection, cultures should be obtained from each of these foci for the preparation of autogenous vaccines and bacterial filtrates. A vaccine and filtrate are prepared from each different organism isolated from the cultures. These autogenous vaccines and filtrates are tested intradermally on the patient, and those that react are used for treatment. Treatments are given at weekly intervals, starting with a small dose, which is gradually increased according to the local reaction and the condition of the patient. Each dose should be regulated so as to produce a satisfactory

local reaction persisting on the arm for forty-eight hours, without any focal or constitutional symptoms. I have frequently seen an aggravation of the arthritis follow too rapid an increase in dosage. Crowe<sup>6</sup> of England, who devotes his entire time to the study of arthritis, claims that the right vaccine in the proper dosage is the ideal form of treatment for this disease. Vaughan<sup>7</sup> reports complete or partial relief from autogenous vaccine treatment alone, in 69 per cent of a series of one hundred cases of arthritis. Both Crowe and Vaughan stress the importance of small doses in the vaccine desensitization treatment of arthritis.

#### SUMMARY

Arthritis is frequently due to sensitization of the joint tissues, either to various foods, or to bacterial products liberated from foci of infection elsewhere in the body. The treatment consists of elimination of all offending foods, removal of all foci of infection, and specific bacterial desensitization.

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1801 EYE STREET, N. W.

## PHOSPHATASE IN HETEROTOPIC BONE FORMATION FOLLOWING TRANSPLANTATION OF BLADDER MUCOSA\*

E. M. REGEN, M.D., AND WALTER E. WILKINS, PH.D., NASHVILLE, TENN.

HUGGINS<sup>1</sup> has shown that "bone forms around transplants of bladder, ureter, and renal pelvis epithelium to certain parietal fascias (the rectus sheath, fascia lata, subcutaneous tissue) and to muscle and synovial membrane in the dog." He reported that this is "true spongy bone with haversian canals containing fibrous and hemopoietic bone marrow." In a later study<sup>2</sup> he found that "in the presence of bone so formed the ratio of phosphatase activity to dry weight was very high, at least 2.5 to 8 times higher than the maximum, and from 10 to 20 times the mean values obtained for the control tissue without bone."

During the course of some other studies on bone phosphatase, we carried out an experiment similar to the phosphatase studies of Huggins, confining our studies to the bladder mucosa and the sheath of the rectus abdominus muscle using dogs as the experimental animals.

*Procedure.*—Under ether anesthesia and with aseptic precautions, a 2-inch incision was made in middle lower abdominal region, the urinary bladder exposed, an incision was made through the muscular coat down to the mucosa, the muscle was carefully pushed back until an area of the mucosa large enough to be grasped with the forceps was exposed, and the desired amount of mucosa was thus practically freed from muscle and excised. The incision in the bladder was closed with silk sutures. The sample of mucosa thus obtained was divided into two parts in such a manner that the exact weight of one would furnish a basis for the estimation of the weight of the other. In two instances both samples were weighed on a torsion balance, the "hook" of which had been sterilized with the operating instruments. One sample was sutured to the rectus sheath with silk and the abdominal incision closed with silk. The other sample was ground with (40 mesh) carborundum and extracted with water (containing  $\text{CHCl}_3$ ) for twenty-four hours. It was then made to the smallest convenient volume with water and centrifuged. Phosphatase determinations were made on the supernatant according to the technique described by Jenner and Kay, 1932.<sup>3</sup> From the phosphatase activity of this sample that of the implant was calculated. These values are shown in Table I. After eleven to one hundred and eleven days the old incisions were reopened. In most instances cysts had formed and these were removed. In the first four cases these were cut in half, one portion being used for microscopic sections and the other for phosphatase determination. In

\*From the Departments of Surgery and Biochemistry, Vanderbilt University Medical School.

Received for publication, April 19, 1934.

Part of the expense of this investigation was borne by a grant from the Division of Medical Science of the Rockefeller Foundation.

TABLE I  
SYNTHESIS OR MOBILIZATION OF PHOSPHATASE DURING HETEROTOPIC OSSIFICATION RESULTING FROM TRANSPLANTATION OF BLADDER MUCOSA ON SHEATH OF RECTUS

(1)	(2)	(3)	(4)	PHOSPHATASE IN REMOVED SAMPLE	$\frac{(4)}{(3)}$	P'ASE IN BLOOD PLASMA PER 100 C.C.	P'ASE IN CYSTIC FLUID	DEFINITE BONE FORMATION	INFECTION
DOG	DAYS TRANSPLANT REMAINED IN DOG	WT. OF TRANSPLANT (APPROX.)	PHOSPHATASE IN TRANSPLANT	units		units	units		
1-98	22	mg. 130	0.134	0.164	1.2	10.9	-	No	Yes
2-85	20	67	0.042	1.500	35.7	7.5	-	Yes	Yes
3-42	49	195	0.265	2.380	9.0	3.6	-	Yes	No
4-2	11	145	0.164	0.194	1.2	11.6	-	No	No
5-23	111	180	0.092	2.400	13.0	-	-	Yes	No
6-70	27	588*	0.735	0.274	0.4	-	-	No	No
7-75	15	332*	0.170	1.13	6.7	-	-	Yes	No
8-7	56	777	2.0	9.3	1.2	-	-	Yes	No
9-6	56	825	1.60	1.34	1.2†	-	1.9	Yes	No

\*Exact weight.

†This bone was not used for phosphatase activity. These values were obtained from the cystic fluid alone.

the next four cases the whole cyst was ground in carborundum, extracted and made to volume as was the mucosa, and phosphatase determinations made on aliquots. In the last case, phosphatase determinations were made on samples of the cystic fluid only, the bone being cleaned and preserved. In this case the cystic fluid alone contained more phosphatase (phosphatase activity) than the original transplant.

When Dog 6 was reopened for removal of the transplant, it was found embedded in the rectus abdominal muscle entirely out of contact with the rectus sheath. No bone was found and the amount of phosphatase (phosphatase activity) found in it was less than when it was implanted in the dog twenty-seven days before.

Histologic sections were made from samples from the first four dogs only. In no case were complete serial sections made and thus it was not possible to ascertain beyond any doubt that no bone was formed in Dogs 1, 4 and 6, although on careful gross inspection no bone could be found.

The amount of bone formed does not seem to vary directly with the size of the sample of implanted bladder mucosa, although the largest implant made resulted in the largest bone (Dog 9-6). This may be due to the fact that the implants did not remain exactly in the original position, thus exposing varying areas to the rectus sheath. In any case the amount of the enzyme as measured by its activity is decidedly increased when bone appears. This is in agreement with the results of Huggins.<sup>2</sup>

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# A NOTE ON THE MENSTRUAL INFLUENCE ON BLOOD MORPHOLOGY\*

ALLAN WINTER ROWE, AND MARY C. GUAGENTY, BOSTON, MASS.

IN THE course of a more extended investigation on various factors influencing blood morphology, a small group of consecutive analyzes were made to ascertain if there were a significant influence from menstruation on the blood picture. Four healthy athletic young women formed the nucleus of the group. To these were added 2 more equally athletic girls but with proved dysfunctional states of the pituitary associated with irregularity in the periods with shortened interval and normal or increased flow. Subject "W" had normal periods at from seven- to fourteen-day intervals; Subject "R" slightly more profuse periods at intervals of from eighteen to twenty-eight days. The essential basic data are given in Table I.

TABLE I  
BASIC DATA

DATUM	NORMAL				PITUITARY	
	P.	D.	G.	K.	W.	R.
Age (yr.)	19	20	20	20	21	20
Height (cm.)	158.4	160.0	158.8	159.7	157.0	166.8
Weight (kg.)	55.0	60.5	56.4	58.2	53.2	66.8
Deviation (%)	-7.0	+1.0	±0.0	+3.0	-2.0	+8.0
Lung volume (%)	+7.0	+13.0	+7.0	+16.0	-2.0	-1.0
Total nitrogen (gm.)	7.1	9.1	7.5	12.5	5.6	10.3
Residual nitrogen (%)	8.4	7.4	9.1	6.7	7.2	8.7
Basal rate (%)	±0.0	-6.0	-10.0	-6.0	(-9.0)	-19.0
Sugar tolerance (%)	±0.0	±0.0	±0.0	±0.0	-75.0	-50.0

The data from the normal group attest the warrant of their designation. With Subject "W" we were unable to get a satisfactory basal rate, her true level falling materially below that recorded. Her presenting difficulty was promptly corrected by the therapeutic approach with pituitary medication. The individual menstrual habits are recorded in Table II.

TABLE II  
MENSTRUAL DATA

SUBJECT	ONSET	INTERVAL	PERIOD	NAPKINS
<i>Normal</i>				
P	13	28	7	5
D	13	25-30	3-4	3-4
G	12	28	4	3-4
K	13	28	4	3-4
<i>Pituitary</i>				
W	11	7-14	3-4	2-3
R	12	18-28	4	4-5

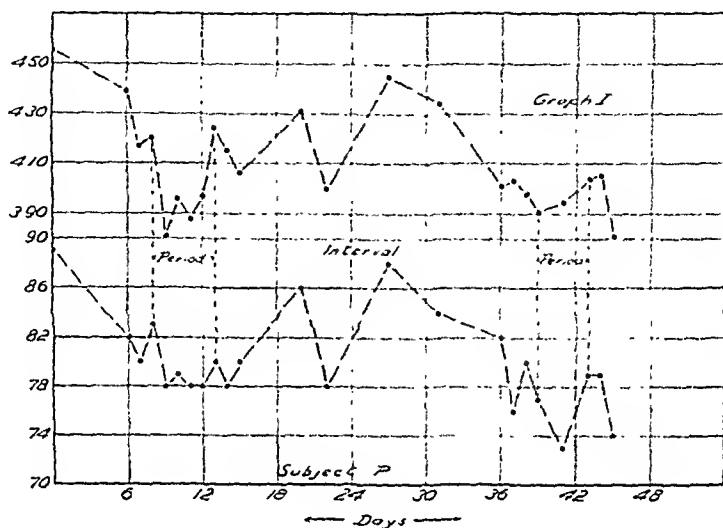
\*From the Evans Memorial, Massachusetts Memorial Hospitals.

Received for publication, April 7, 1934.

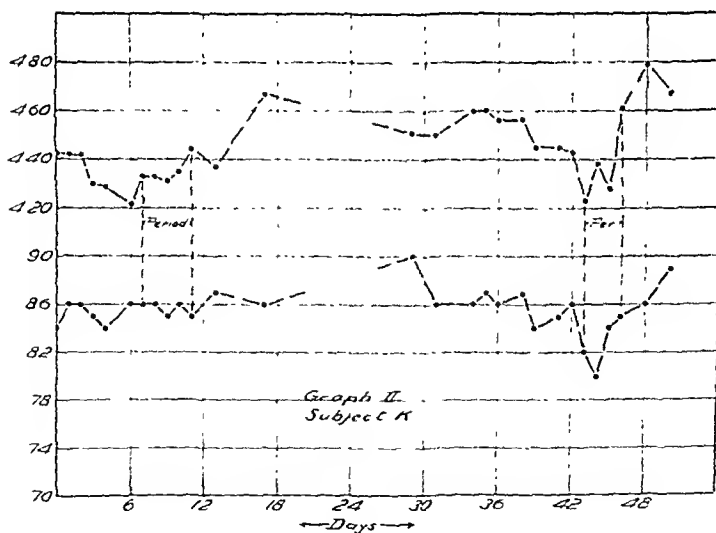
Presented before the Biological Chemical Section of the American Chemical Society, Washington, March, 1933.

Subject "P" had long and ample periods; the others conform more nearly to the conventional mode.

All counts were made in the early morning with the subjects in a fasting state. So far as was possible, representative counts were made during the interval between periods together with daily records from two days before to



Graph I.

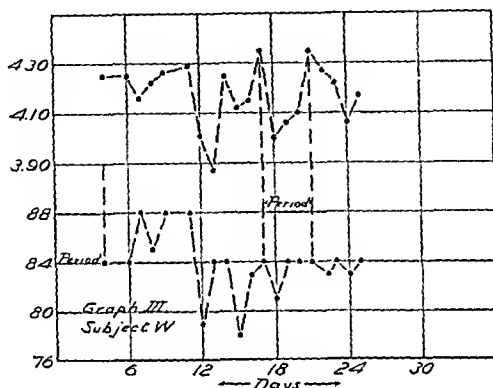


Graph II.

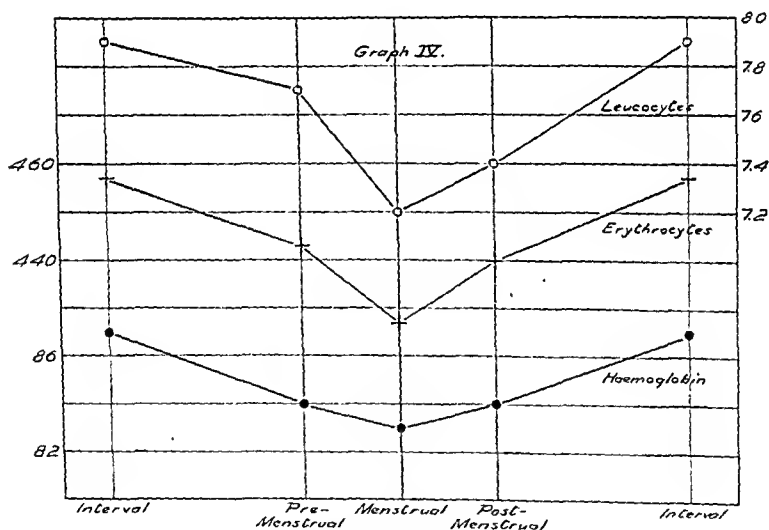
two days after the period. Bloods were taken before 8 P.M.; the hemoglobin estimated by the Dare and Newcomer procedures and red and white counts made in duplicate by the usual procedures using a standardized Levy (double Neubauer ruling) counting chamber. For the differential counts, duplicate smears were prepared with Wright's stain and 200 cells counted with each,

All of the curves show irregularities unassociated with any known influence. The general picture of the changes in hemoglobin and erythrocytes can best be shown graphically (Graph I).

This normal girl shows a definite drop of both components in the first period recorded; both the pre- and postmenstrual intervals reflect this depression. The second period is perhaps less well marked; transitory recovery of the erythrocyte



Graph III.



Graph IV.

count begins with the period. It will be noted that she was slightly delayed beyond her usual habitude.

Subject "K," whose record is given in Graph II, shows a variant of the preceding in that the first period demonstrates no change in hemoglobin and a slight rise in the red cell count during the first period. In the second, there is a gradual depression reaching culmination with inversion of the curve during the time of flowing. She, too, shows a protracted intermenstrual interval.

This is a partial record of the pituitary Subject "W" with marked fluctuations during the interval and but minor change in the hemoglobin during the

period. The erythrocytes demonstrate a sharp drop in the middle of the period with, however, the two highest and equal values recorded on the first and last days of the flow. The picture is one of a lack of normal control and may be felt to reflect the drain of her excessive and unregulated catamenia.

While the subjects were examined in a fasting state, it was impossible to arrange for basal examinations. We did not find, however, those high leucocyte counts following physical activity which have been frequently recorded in the literature. With but occasional minor exception, all of the values fell within the usual conventional normal range. The total leucocyte count showed much the same fluctuations and in perhaps a slightly more marked degree as were exhibited by the hemoglobin and red cells. The differential counts were seemingly unaffected by the catamenial influence. Taking the averages of all records for the four intervals determined by the presence or absence of menstruation, the results are given in Table III.

TABLE III  
DIFFERENTIAL LEUCOCYTE COUNT

CELL	INTERVAL	PREMENSTRUAL	MENSTRUAL	POSTMENSTRUAL
Neutrophiles	52.0	50.0	50.0	51.0
Lymphocytes	39.0	38.0	39.0	38.0
Endothelial leucocytes	6.5	8.5	7.5	7.5
Eosinophiles	2.0	3.0	3.0	3.0
Basophiles	0.5	0.5	0.5	0.5

The lymphocytes are relatively slightly high, the neutrophiles correspondingly low. While the method of averages conceals individual variation, these figures represent the group and show no evidence of menstrual influence on the differential picture.

Still using average values, the general menstrual influence can be shown schematically.

All of the factors show a downward tendency during the flow with gradual recovery after its termination. The average change is not great, and the individual records of this small group would seem to indicate that none of them had a characteristic pattern. With Subject "W," the excessive blood loss seemed to be associated with some lack of control in the regeneration processes.

# FACTORS INFLUENCING THE SEDIMENTATION RATE OF ERYTHROCYTES\*

THOMAS H. CHERRY, M.D., F.A.C.S., NEW YORK, N. Y.

FROM the time that Fåhræus<sup>1</sup> in 1921 first elucidated his hypothesis "that the plasma contains a substance responsible for the increased sedimentation of erythrocytes" and "that the increased rate was due to their increased agglutination which in turn was dependent on the properties of the plasma" to the present day, many investigators have discussed this phenomenon in the literature. The variance of their findings and contentions encouraged me to attempt to evaluate the test by a combination of clinical and laboratory data. The data herein assembled are the result of nine years' observation of the sedimentation test done in an indiscriminate group of both hospitalized and ambulatory patients. The technic employed was the Friedlaender modification of the original Linzenmeier method.

To establish a normal basis for comparison, the sedimentation rate was performed on twelve healthy physicians and laboratory workers. The following tabulated results correspond with the normals given by Linzenmeier,<sup>2</sup> Flores,<sup>3</sup> Geppert,<sup>4</sup> Friedlaender,<sup>5</sup> and Baer and Reis.<sup>6</sup>

TABLE I  
SEDIMENTATION TIME OF NORMAL INDIVIDUALS

	2 HOURS	3 HOURS	4 HOURS	5 HOURS	6 HOURS
6 men	1	2	1	1	1
6 women	1	1	1	1	2

Most observers agree that the chief influence on the sedimentation of erythrocytes is an inherent factor in the plasma, and even in the days of Galen,<sup>7</sup> when he described the *crusta phlogistica*, it was recognized that standing blood would settle in two layers, at different rates, as first noted by Hunter.<sup>8</sup> In 1897, Pfeiffer<sup>9</sup> studying on the formation of the "buffy coat" said, "an increase in the sedimentation of erythrocytes was due to an increase in agglutination, and that an increase in the fibrinogen factor might cause the corpuscles to coagulate more rapidly and in this way further the agglutination." Dogiel,<sup>10</sup> Hayem,<sup>11</sup> and Valentin<sup>12</sup> all found that an increase in sedimentation was generally caused by increased agglutination. These facts suggested the channel of laboratory investigation of the plasma fraction, and a partition was therefore done.

To establish a group of controls, fractions were done upon five persons having a normal sedimentation rate.

\*Received for publication, April 4, 1931.

Read before the New York Pathological Society, November 14, 1931.

This study was made possible by a grant from the Alice L. Filbert Fund.

The foregoing Tables III and IV, indicate that I have arbitrarily divided them into a "Fast Group" (Table III) ranging in time from five to sixty minutes and a "Slow Group" (Table IV) ranging from sixty to three hundred sixty minutes. In analyzing these tables very little alteration in the fractions is apparent in either the slow or fast groups, as compared with the controls or normals.

In the "fast group" there was an increase in the fibrin nitrogen, the average (Table V) being 0.18 as compared with the average normal of 0.06, and in the "slow group" it averaged 0.11. These results confirm the observations of Cordua and Hartmann<sup>13</sup> and Frisch and Starlinger.<sup>14</sup>

TABLE II  
PARTITIONS UPON NORMAL INDIVIDUALS

PATIENT	TOT. N.	N. P. N.	PROT. N.	FIBRIN N.	EUGLB. N.	1 PSDO. GLN.	2 PSDO. GLN.	TOT. GLN.	ALB. N.	S. T.
1	1.16	0.02	1.14	0.07	0.15	0.19	0.15	0.49	0.58	5 hr. +
2	1.08	0.03	1.05	0.03	0.08	0.18	0.16	0.42	0.60	5 hr. +
3	1.10	0.04	1.06	0.01	0.19	0.20	0.08	0.47	0.58	3 hr. +
4	1.19	0.01	1.18	0.12	0.16	0.12	0.19	0.47	0.71	6 hr.
5	1.19	0.02	1.19	0.10	0.27	0.18	0.15	0.60	0.49	3 hr.

TABLE III  
PARTITIONS UPON DISEASED INDIVIDUALS (FAST GROUP)

PATIENT	TOT. N.	N. P. N.	PROT. N.	FIBRIN N.	EUGLB. N.	1 PSDO. GLN.	2 PSDO. GLN.	TOT. GLN.	ALB. N.	S. T.
1	1.27	0.03	1.24	0.23	0.32	0.24	0.10	0.66	0.35	5 min.
2	1.49	0.30	1.19	0.15	0.27	0.29	0.00	0.55	0.49	5 min.
3	1.34	0.01	1.33	0.26	0.26	0.25	0.17	0.81	0.26	15 min.
4	1.11	0.03	1.08	0.21	0.27	0.24	0.07	0.58	0.29	15 min.
5	1.02	0.04	0.98	0.16	0.20	0.20	0.14	0.54	0.28	15 min.
6	1.28	0.22	1.16	0.13	0.18	0.19	0.13	0.50	0.43	15 min.
7	1.37	0.23	1.14	0.24	0.13	0.19	0.08	0.58	0.32	15 min.
8	1.38	0.27	1.11	0.15	0.23	0.17	0.16	0.56	0.40	15 min.
9	1.37	0.08	1.29	0.29	0.43	0.18	0.10	0.77	0.29	20 min.
10	1.02	0.02	1.00	0.18	0.29	0.06	0.11	0.14	0.36	25 min.
11	1.44	0.03	1.41	0.08	0.34	0.35	0.13	0.82	0.51	25 min.
12	1.35	0.03	1.32	0.33	0.25	0.28	0.10	0.63	0.36	40 min.
13	1.18	0.04	1.14	0.12	0.16	0.17	0.14	0.47	0.55	45 min.
14	1.04	0.02	1.02	0.11	0.18	0.18	0.11	0.47	0.44	50 min.
15	1.41	0.01	1.40	0.20	0.30	0.28	0.14	0.72	0.48	50 min.
16	1.13	0.01	1.12	0.13	0.15	0.18	0.19	0.52	0.47	60 min.

TABLE IV  
PARTITIONS UPON DISEASED INDIVIDUALS (SLOW GROUP)

PATIENT	TOT. N.	N. P. N.	PROT. N.	FIBRIN N.	EUGLB. N.	1 PSDO. GLN.	2 PSDO. GLN.	TOT. GLN.	ALB. N.	S. T.
1	1.44	0.02	0.42	0.39	0.40	0.25	0.15	0.80	0.23	70 min.
2	1.13	0.08	1.05	0.13	0.11	0.22	0.11	0.44	0.48	85 min.
3	1.13	0.01	1.12	0.15	0.11	0.31	0.10	0.52	0.45	180 min.
4	1.04	0.03	1.01	0.01	0.05	0.24	0.08	0.37	0.63	180 min.
5	1.19	0.02	1.19	0.10	0.27	0.18	0.15	0.60	0.49	180 min.
6	1.10	0.04	1.06	0.01	0.19	0.20	0.08	0.47	0.58	180 min.
7	1.16	0.02	1.14	0.07	0.15	0.19	0.15	0.49	0.58	300 min.
8	1.18	0.03	1.05	0.03	0.08	0.18	0.16	0.42	0.60	300 min.
9	1.19	0.01	1.18	0.12	0.16	0.12	0.19	0.47	0.71	360 min.

The average euglobulin nitrogen was 0.17 in the normals, in the "fast group" 0.26, and in the "slow group" 0.12.

The average total globulin nitrogen in the normals was 0.49, 0.58 in the "fast group," and 0.15 in the "slow group." The increase in the globulin content in the fast sedimentation group parallels the results on Salomon.<sup>15</sup>

Table V shows that there is an appreciable increase in the fibrin nitrogen, euglobulin nitrogen, and the globulin nitrogen content in fast sedimentation cases, with only a slight increase in the slow cases, and a practically negligible increase in the pseudoglobulin 1 and 2 in both slow and fast.

TABLE V  
AVERAGES OF TABLES II, III AND IV

	CONTROL	FAST	SLOW
Fibrin N.	0.06	0.18	0.11
Euglobulin N.	0.17	0.26	0.12
Pseudo Gl. 1 N.	0.17	0.21	0.21
Pseudo Gl. 2 N.	0.14	0.11	0.13
Globulin N.	0.49	0.58	0.51
Albumin N.	0.59	0.38	0.53

TABLE VI  
THE EFFECT OF PROTEIN INGESTION UPON THE SEDIMENTATION TIME

	SED. TIME	N. P. N. MG./100	UREA N.	AMINO N.
Control	180	30.2	17.3	4.2
First hour	180	38.3	15.6	6.3
Second hour	180	37.3	16.8	8.4
Third hour	180	41.7	21.0	7.7
Control	50	24.6	10.3	5.6
First hour	35	26.5	10.5	4.9
Second hour	100	33.4	11.4	10.5
Third hour	45	39.0	12.8	4.9
Control	25	31.5	12.5	4.2
First hour	25	33.0	13.2	6.3
Second hour	20	33.0	14.0	7.0
Third hour	20	33.0	13.5	4.9
Control	30	23.9	12.8	7.0
First hour	20	25.2	11.3	7.7
Second hour	25	27.6	13.5	7.7
Third hour	25	29.3	13.2	9.1

The albumin nitrogen shows an average decrease in the fast cases with practically no change in the slow cases. This agrees with the findings of Mikulicz-Radecki.<sup>16</sup> This fall in the albumin nitrogen content in the fast cases can be accounted for by Nature's effort to compensate for the rise in fibrin nitrogen.

With this laboratory data in mind, an attempt was made to determine the effects of protein ingestion on the sedimentation rate.

A group of patients under study were each fed 150 gm. of lean meat and six ounces of water, and the sedimentation rate, nonprotein nitrogen, urea, and amino nitrogen, were determined each hour for the succeeding three hours. A control specimen was taken in each instance (Table VI).

In Case 1 (Table VI) all the fractions were progressively increased over the period of investigation with no change in the sedimentation rate. Cases 2,

3, and 4, which have been classified as "rapid," do not show any alteration in the sedimentation rate, in spite of the apparent increase in all fractions over the period. Therefore, it may be said that increasing the protein fractions does not alter either the slow or fast rates. Following up this trend of thought, the sedimentation rate of three cases of definite renal involvement was determined (Table VII).

TABLE VII  
THE SEDIMENTATION RATE IN NEPHRITICS

1. Urine protein ++	Blood urea 22.7	N. P. N. 39.4		S. R. 180 min.
2. Urine protein +	Blood urea 30.0	N. P. N. 55.6		S. R. 51 min.
3. Urine protein ++++	Blood urea 124.5	Creatinine 21	Amno N. 49	S. R. 5 min.

After a review of all the findings regarding the nitrogen partition's influence on the sedimentation rate, it seems justifiable to state that in the face of such wide variations, no reliance can be placed on its influence on the sedimentation time.

To determine what influence glucose had on the sedimentation rate, a group of six individuals with normal blood sugars ranging from 0.078 to 0.142 mg./100 were selected for the glucose tolerance test. Each was given the standard amount of 1.74 gm. glucose per kilo of body weight, with sugar estimations and sedimentation rate readings at one- and two-hour intervals (Table VIII).

TABLE VIII  
EFFECT OF GLUCOSE INGESTION UPON THE SEDIMENTATION RATE

PATIENT	CONTROL		FIRST HOUR		SECOND HOUR	
	S. R.	SUGAR PER CENT	S. R.	SUGAR PER CENT	S. R.	SUGAR PER CENT
1	20	0.113	20	0.187	20	0.182
2	20	0.109	20	0.195	20	0.175
3	45	0.142	45	0.258	45	0.340
4	120	0.097	155	0.117	150	0.103
5	120	0.078	30	0.142	85	0.138
6	155	0.095	85	0.158	110	0.110

It will be observed that in the fast cases the sedimentation rate remained constant despite an increased sugar percentage, whereas in the slow group it showed a decrease in the rate along with an increase in the sugar percentage. In an attempt to correlate these findings, a group of recognized diabetic patients were studied (Table IX).

TABLE IX  
SEDIMENTATION RATE IN DIABETIC PATIENTS

	PATIENT	BLOOD SUGAR	SEDIMENTATION RATE
1	G.	0.445	33 min.
2	S.	0.156	51 min.
3	G.	0.297	23 min.
4	A.	0.240	108 min.
5	S.	0.156	382 min.
6	N.	0.193	205 min.
7	N.	0.096	357 min.



In the glucose tolerance control patients, it is apparent that in Cases 4, 5, and 6, or fast sedimentation rate, the higher the sugar percentage, the slower the rate; this seems to be the reverse in cases of clinical hyperglycemia.

The effect of the ingestion of food on the stability of the blood was shown by Rourke and Plass<sup>17</sup> to be negligible and confirms De Courey's<sup>18</sup> observations.

Although the blood plasma has been credited with containing the necessary "something" which influences the sedimentation rate of the erythrocytes, it is certain that the cells themselves play a most important part. Groedel and Hubert<sup>19</sup> state that the erythrocytes are the most important single blood component in the sedimentation rate. In cases of high hemoglobin and a normal red blood cell count the rate is usually normal or slightly slowed. They also noticed that an increased rate is more common in blood with low hemoglobin, differing from Westergren,<sup>20</sup> who claimed that the sedimentation rate is influenced chiefly by the hemoglobin content. Rubin's and Smith's<sup>21</sup> findings in this relation were in accord with those of Groedel and Hubert.

Tables X and XI refer to a study of eighteen patients upon whom red blood cell count, hemoglobin, and sedimentation time were taken. They were divided

TABLE X  
RELATION OF RED CELL COUNT, HEMOGLOBIN AND SEDIMENTATION TIME  
(FAST GROUP)

NAME	R. B. C.	HEMOGLOBIN	SEDIMENTATION TIME
1	1,950,000	40%	1 min.
2	3,240,000	54%	5 min.
3	3,300,000	60%	13 min.
4	3,680,000	86%	15 min.
5	1,790,000	32%	15 min.
6	2,240,000	50%	15 min.
7	2,800,000	40%	15 min.
8	3,520,000	64%	20 min.
9	2,230,000	50%	20 min.
10	3,560,000	67%	25 min.
11	3,670,000	70%	30 min.
12	3,330,000	70%	30 min.
13	4,220,000	67%	45 min.

TABLE XI  
RELATION OF RED CELL COUNT, HEMOGLOBIN AND SEDIMENTATION TIME  
(SLOW GROUP)

NAME	R. B. C.	HEMOGLOBIN	SEDIMENTATION TIME
1	4,600,000	84%	60 min.
2	3,700,000	58%	70 min.
3	2,940,000	50%	70 min.
4	2,200,000	32%	85 min.
5	4,725,000	74%	100 min.

into the "fast" and "slow" groups according to the sedimentation time. As can be seen in the "fast" group, where all the rates of blood were below sixty minutes, when there was a low red cell count and hemoglobin, a fast sedimentating blood was usually present. However, a sufficient number of exceptions occur to prevent definite conclusions being drawn.

Table X shows hemoglobin varying from 32 to 86 per cent and red blood cells 1,790,000 up to 4,220,000 with the sedimentation rates varying from one minute to forty-five minutes.

Table XI shows the hemoglobin from 32 to 84 per cent and the red blood cells from 2,200,000 to 4,600,000, and the sedimentation rate from sixty to one hundred minutes.

There does not seem to be any consistency in relationship or parallelism between the hemoglobin, red blood cells and sedimentation time in either group. This can be seen by comparing Case 10 of the "fast" group (with hemoglobin 32 per cent, red blood cells 1,790,000 and sedimentation time of fifteen minutes) with Case 17 of the "slow" group (with hemoglobin 32 per cent, red blood cells 2,200,000 and a sedimentation time of eighty-five minutes); and again, Case 2 in the "fast" group (hemoglobin 86 per cent, red blood cells 3,680,000, sedimentation time fifteen minutes), with Case 15 in the "slow" group (84 per cent hemoglobin, red blood cells 4,600,000, and sedimentation time of sixty minutes). This discrepancy is noted throughout. Variations occurred sufficiently often in this series of pathologic states to prevent establishing a definite ratio between the red blood cells, hemoglobin, and sedimentation time. These findings agree with the observation of Hunt,<sup>22</sup> who claims that in normal bloods the red blood cells, hemoglobin, and sedimentation rate show a direct relationship, and also believes that as the blood count decreases the sedimentation rate increases, but no definite parallelism exists.

It is an established fact that the percentage of fibrin of the blood in simple anemia, aplastic anemias, and polycythemia is found almost consistently within normal limits. In the leucemias and pseudoleucemias there is only slight, if any, increase. In the primary anemias there is a diminished fibrin. Gram<sup>23</sup> found an increase sedimentation of erythrocytes in anemias that closely paralleled the decrease in the red blood cells, and that the fibrin values did not increase unless there was secondary infection.

Any marked variation of the cell count, either above or below normal limits, affects the sedimentation rate. This was demonstrated in two cases of polycythemia (Table XII) and five cases of secondary anemia (Table XIII) (with established causes) that were studied.

TABLE XII  
SEDIMENTATION TIME IN TWO CASES OF POLYCYTHEMIA

NAME	R.B.C.	HEMOGLOBIN	S. T.	R.B.C. VOL.	PLASMA
1	8,200,000	132%	18 hours	71%	29%
2	8,150,000	110%	5 hours	72%	28%

TABLE XIII  
SEDIMENTATION TIME IN FIVE CASES OF SECONDARY ANEMIA

PATIENT	R.B.C.	HEMOGLOBIN	SEDIMENTATION TIME
1	3,240,000	54%	5 min.
2	2,350,000	55%	6 min.
3	2,350,000	32%	23 min.
4	2,240,000	50%	22 min.
5	3,590,000	58%	43 min.

The sedimentation rate in relationship to cell volume and total solids was next studied (Table XIV).

TABLE XIV

RELATION TO THE SEDIMENTATION TIME TO CELL VOLUME AND TOTAL SOLIDS

PATIENT	SEDIMENTATION TIME	R.B.C. VOL. PER CENT	TOTAL SOLIDS PER CENT
1	5 min.	20	16.0
2	15 min.	29	18.0
3	25 min.	36	22.3
4	300 min.	45	22.8
5	18 hours	71	21.0
6	5 hours	72	28.0

"In blood the sedimentation rate of the erythrocytes is, roughly, inversely proportional to the cell volume, provided other factors are unchanged." This was stated by Rourke and Plass<sup>17</sup> and emphasized by additional workers (De Courey<sup>18</sup> Gram,<sup>23</sup> and Rubin and Smith<sup>21</sup>). Rourke and Plass, in their experimental work on the effect of blood dilution on the sedimentation rate, concluded that it is very important that the result of a sedimentation test should not be interpreted in terms of infection or of other tissue damage, without consideration being paid to the concentration of the blood (cell volume).

A study of the red blood cells and sedimentation rate led to the investigation of the relationship which exists between the leucocytes and sedimentation rate (Table XV). No particular class of pathologic conditions was selected; hospital cases were taken at random.

TABLE XV

RELATION OF THE SEDIMENTATION TIME TO LEUCOCYTE COUNT (FAST GROUP)

PATIENT	SEDIMENTATION RATE	W.B.C.
1	5	10,000
2	5	10,200
3	5	21,300
4	5	25,250
5	5	12,500
6	5	17,000
7	10	7,000
8	10	19,950
9	15	10,800
10	15	9,800
11	15	22,000
12	15	11,700
13	15	11,400
14	15	14,000
15	15	9,800
16	15	13,000
17	15	12,400
18	15	10,200
19	15	33,550
20	15	25,600
21	15	17,500
22	20	11,400
23	20	20,000
24	20	11,200
25	25	10,000
26	25	27,000
27	25	8,000
28	25	25,200
29	30	8,800
30	35	9,250
31	50	8,000

According to Halliburton,<sup>24</sup> Lacksewitz,<sup>25</sup> Berggrün,<sup>26</sup> and Dochez<sup>27</sup> many infections and diseases such as pneumonia, tuberculosis, pleurisy, peritonitis, acute rheumatic fever, erysipelas, and scarlatina show an increase of fibrin in the blood. Pfeiffer<sup>9</sup> in reporting his extensive investigations of the fibrin content of the blood in disease, stated that in general the fibrin was increased. He also noted that within the increased fibrin there was a leucocytosis, and with a decrease in the leucocytes a normal fibrin content existed.

The high leucocyte counts shown in Tables XV and XVI were from specimens taken from a diversified group of acutely ill hospital patients suffering from meningitis, pleurisy, abscesses in the lungs and peritoneal cavity, pneu-

TABLE XVI  
RELATION OF SEDIMENTATION RATE TO THE LEUCOCYTE COUNT (SLOW GROUP)

PATIENT	SEDIMENTATION RATE	W.B.C.
1	70	5,900
2	70	11,200
3	85	7,800
4	85	9,600
5	115	11,160
6	115	9,850
7	120	10,300

TABLE XVI  
FAST GROUP

PATIENT	SEDIMENTATION RATE BEFORE	SEDIMENTATION RATE AFTER WASHING IN NORMAL SALINE AND REPLACING IN OWN PLASMA
1	45 min.	35 min.
2	35 min.	35 min.
3	20 min.	25 min.
4	15 min.	15 min.
5	25 min.	40 min.
6	10 min.	10 min.
7	35 min.	23 min.

monia, erysipelas, peritonitis, and salpingitis. They verify the reports of other investigators that leucocytosis by its increase in fibrin content increases sedimentation rate.

It was thought that some information of value might be obtained by crossing the elements of fast sedimentating blood with the elements of slow sedimentating blood, using the same blood groups. Hunt observed that by exchanging so-called slow cells with fast plasma he obtained practically the same rate of the cells. If the cells of this blood were transferred to the slow plasma, the results were almost the same as when the original cells were in the plasma. He said, "as this phenomena occurred quite consistently in all samples of blood obtained from patients who were in compatible groups, it would seem that the substance controlling the sedimentation of the erythrocytes is contained in the plasma."

Rourke and Plass<sup>17</sup> have shown that when centrifuging for as long as twenty minutes at 2,500 r.p.m. the stability of the remixed blood is not different from that of the freshly drawn sample.

In order to determine what effect washing the cells in normal saline solution and replacing them in their own and other sera would have, they were first centrifuged and then replaced in their own plasma. The results were practically the same as the original sedimentation rate. The erythrocytes were then centrifuged out of their plasma, washed in normal saline, and replaced in their own plasma. Tables XVI and XVII show the results.

TABLE XVII  
SLOW GROUP

PATIENT	SEDIMENTATION RATE BEFORE	SEDIMENTATION RATE AFTER WASHING IN NORMAL SALINE AND REPLACING IN OWN PLASMA
1	240 min.	180 min.
2	240 min.	420 min.
3	180 min.	180 min.
4	180 min.	180 min.
5	180 min.	210 min.

In twelve cases there was only one blood in which the rate was appreciably decreased (Case 2 of the slow group) and one in which the rate was increased (Case 5 of the fast group). The conclusions from these findings can logically be that changes in the physical environment of the erythrocytes have very little influence upon the sedimentation rate, and can be practically ignored when cross sedimentation tests were made.

The "fast red cells" after centrifuging and washing with normal saline were next crossed with "slow plasma." The result of seventeen such experiments is shown in Table XVIII.

TABLE XVIII  
"FAST" RED BLOOD CELLS CROSSED WITH "SLOW" PLASMA

R.B.C.	PLASMA	CROSSED	PER CENT CHANGE IN TIME
5	120	5	0
10	240	300	+2900
10	85	20	+ 100
15	120	50	+ 233
15	120	30	+ 100
15	300	120	+ 700
15	180	180	+1100
15	240	420	+ 833
20	140	35	+ 75
20	180	240	+1100
25	360	360	+1340
25	180	300	+1100
30	240	240	+ 700
30	180	45	+ 50
35	180	360	+ 928
45	240	420	+ 833
45	120	55	+ 22

A tremendous change varying from 22 per cent to 2,900 per cent in slowing of the rate is noted here (Table XVIII).

This experiment was then repeated, using the reverse process of mixing slow red cells with fast plasma. The result in fourteen cases is shown in Table XIX.

TABLE XIX  
 "SLOW" RED BLOOD CELLS CROSSED WITH "FAST" PLASMA

R.B.C.	PLASMA	CROSSED	PER CENT CHANGE IN TIME
85	10	35	-59
120	15	30	-75
120	20	55	-54
120	5	20	-83
120	15	30	-75
140	20	50	-64
180	35	35	-80
180	20	25	-80
180	25	10	-94
240	45	70	-8
240	30	95	-60
240	45	70	-8
300	15	15	-95
360	25	60	-85

Here there is a marked acceleration of the rate, varying from 8 per cent to 95 per cent. In reviewing both tables, the findings are in accord with those of other workers. The plasma is the influencing factor and is more pronounced when fast cells are mixed with slow plasma than the reverse, although in the latter instance also, the controlling influence of the plasma is quite marked.

The surface tension in bloods of varying sedimentation rate was next determined. These were tested by the Du Noy Tensionmeter, and the results tabulated in fast and slow groups (Tables XX and XXI).

The average sedimentation rate was twenty-one minutes and the average surface tension was thirty dynes (Table XX).

TABLE XX  
 FAST SEDIMENTATION RATE

PATIENT	SEDIMENTATION RATE	SURFACE TENSION M.M.
1	10	38
2	10	36
3	15	34
4	20	32
5	25	30
6	30	30
7	35	23

TABLE XXI  
 SLOW SEDIMENTATION RATE

PATIENT	SEDIMENTATION RATE	SURFACE TENSION M.M.
1	85	12
2	140	2
3	180	2
4	180	4
5	180	3
6	195	5

The average sedimentation rate was one hundred sixty minutes and the average surface tension was four and six-tenths dynes (Table XXI).

These findings suggest that the faster the sedimentation rate, the greater the surface tension. Since surface tension is due to the fact that the molecules

within a liquid are subjected to equal forces of attraction on all sides, at the surface these forces act only on one side of the molecule and thus tend to pull them inward. This causes the surface to pull itself together so as to occupy the least possible area, and it is this force which constitutes surface tension. Thus it would seem that with an increased molecular concentration in fast sedimentation rate blood, the greater the tension, and vice versa.

Surface tension was then checked upon bloods which had been crossed and the results are shown in Tables XXII and XXIII.

Average slow plasma, 180; average red blood cells, 20; average surface tension, 4.7 m.m.

TABLE XXII

SURFACE TENSION FOLLOWING CROSSING "SLOW" PLASMA WITH "FAST" RED CELLS

PATIENT	SLOW PLASMA	FAST RED BLOOD CELLS	SURFACE TENSION M.M.
1	85	10	15
2	180	20	1
3	180	35	1
4	180	15	6
5	180	10	3
6	195	25	1
7	240	30	6

TABLE XXIII

SURFACE TENSION FOLLOWING CROSSING "FAST" PLASMA WITH "SLOW" RED CELLS

PATIENT	FAST PLASMA	SLOW RED BLOOD CELLS	SURFACE TENSION M.M.
1	10	180	24
2	10	85	24
3	15	180	24
4	25	180	26
5	25	195	30
6	30	240	21
7	35	180	26

Average fast plasma, 21; average slow red blood cells, 180; average surface tension, 25 m.m. (Table XXIII).

From these crossed sedimentation and surface tension readings it seems that where slow plasma and fast cells have been mixed, the surface tension about equals that of the slow sedimentating blood; whereas a mixture of fast plasma and slow cells results in a lowering of the surface tension.

A group of thirty postoperative patients were carefully studied. They were all clean cases, free from pre- and postoperative infections. The sedimentation rate was taken preoperatively, six hours postoperatively, and on the fifth and ninth postoperative days. The chemical blood and urine, as well as a red blood cell and white blood cell count, were simultaneously studied. All of these postoperative cases showed an acceleration of the sedimentation rate and were arbitrarily classed as "fast," some being as rapid as ten minutes and all under sixty minutes. This would seem to coincide with the findings of others, in that it indicates a disturbance of the balance, by autolysis of tissue secondary to surgical trauma.

Greisheimer<sup>25</sup> has shown that the sedimentation rate varies from day to day in healthy individuals, but always within normal limits. This he attributes to the incidental wear and tear of life on body tissue.

In the absence of pyrexia after the initial postoperative rise of temperature subsiding by the fifth day, and the normal leucocyte count, I think we are justified in assuming that the increase in rate is due to a disturbed balance caused by the absorption of tissue destruction, or as has been suggested, that the presence of proteolytic ferments at the site of operation may disturb the fine chemical balance necessary to maintain the equilibrium between the cells and plasma.

Among the gynecologic conditions most often affecting the sedimentation time, pelvic infections, especially the acute inflammations, are most prominent. In the pelvis as in other anatomical sites where infection exists, an increase in sedimentation rate takes place. Many gynecologists have placed great faith in this test as a diagnostic and prognostic aid and have laid considerable stress upon it as a guide as to when to operate for the relief of pelvic infections.

In a former article (Cherry<sup>29</sup>) in which the results of the sedimentation time in pelvic infections were evaluated, it was shown that such an index could not be followed, and that the clinical picture, leucocyte count, and elevation of temperature were more dependable guides.

Benign, uncomplicated pelvic neoplasms as a rule do not show any decided change from normal in the sedimentation rate. However, in the presence of necrobiosis and infection there is an acceleration of the sedimenting velocity, probably due to increase in blood fibrin and decrease in erythrocytes. Benign ovarian neoplasms and fibromyomas showed a normal sedimentation rate in a group of thirty cases. In the presence of secondary anemia, where uterine bleeding is a pronounced symptom, acceleration occurs. These observations agree with those of other workers in this field.

The sedimentation time as a rule is accelerated in all types of malignant disease, irrespective of its location. The acceleration becomes markedly increased in the presence of metastases, necrosis, infections, and anemia. Rubin<sup>30</sup> believes that an increasing sedimentation rate is an indication of the advancement of the disease. This increase in the sedimentation time in cases of cancer can be especially applied to those instances in which infection has not involved either the primary or metastatic growths. My own observations confirm these findings.

All observers agree that during pregnancy erythrocytic sedimentation shows an acceleration after the fourth month of gestation, and is continued into the puerperal state, returning to normal at its termination. Fåhræus considered the acceleration of great value in the diagnosis of early pregnancy. This view is not confirmed by later observers. Hirst and Long,<sup>31</sup> Baer and Reis, Falta<sup>32</sup> all agree that as a diagnostic aid in pregnancy it has no value. There is no doubt that the pregnant state brings about definite changes in the maternal blood, and with these alterations the sedimentation time of red cells is changed. Sakae and Tsutsumi<sup>33</sup> believe that the ratio of the blood volume to the erythrocytes is greater and that the corpuscles agglutinate more rapidly because of



the increase of the globulin and fibrinogen, or because of a decrease in the albumin contents. Gram considered that the lowered percentage of cell volume in the early months of pregnancy and the increase in fibrin in the later months caused the acceleration. It seems that there are several factors influencing this increase of sedimentation time. As pointed out by Bland<sup>34</sup> the anemia accompanying pregnancy would show a lowered cell volume, thus increasing the sedimentation time. He furthermore indicated that as a leucocytosis attends the later months of pregnancy, there is an increase in the fibrin content of the blood which has been believed to hasten the sedimentation velocity.

During the puerperium the sedimentation rate is rapid for the first ten or twelve days, with a gradual decline to normal upon the termination of the involuting process. This process can be likened to the one following operative procedures, where the sedimentation time is always rapid.

#### SUMMARY

1. Plasma partition showed increase in the fibrin nitrogen euglobulin nitrogen, and globulin nitrogen in both slow and fast groups of sedimentating bloods, but was more marked in the fast group.

2. A fall in the albumin nitrogen in the fast group is at the expense of the rise in fibrin and euglobulin nitrogen.

3. No relationship exists between renal involvement and the sedimentation rate.

4. The glucose tolerance test shows a decreased rate with an increase in the blood sugar percentage in controls; the reverse seems to be found in diabetic patients.

5. Normal red blood counts and hemoglobin show a fairly constant ratio with the sedimentation time.

6. Variations of the red blood count from normal affect the sedimentation rate.

7. Leucocytosis increases sedimentation rate, but this is due to the increased fibrinogen content in the blood. Leucopenia gives a normal percentage.

8. Crossed sedimentation adds to the evidence that the plasma is the controlling factor on the sedimentation rate.

#### CONCLUSIONS

It would seem from the foregoing review of the literature, clinical data, and laboratory findings that changes in the blood sedimentation time are caused by disturbing the fine balance between the cellular and fluid elements in the circulation.

Two factors apparently influence the change of rate, cell volume and the variations of fibrin, euglobulin, and globulin in the plasma. It has been shown that these variations from normal occur in so many pathologic states, thus influencing the settling of the erythrocytes, that it is impossible to affix definitely a standard sedimentation rate to any disease.

To rely upon this test alone, without other laboratory data, is unwise. To interpret this test for diagnostic purposes, the cell volume and nitrogen partitions must be determined if it is to be of any value.

From a clinical point of view, the sedimentation test alone is confusing, because individuals react biochemically to disease in different ways, thereby producing variations in rate in the same pathologic state. This has been observed many times; of two persons having the same disease, one will frequently show a sedimentation rate in the fast group and the other in the slow group.

Occasionally it is of aid in obscure cases by suggesting a lead to follow in a new line of investigation.

As a prognostic aid it is of slight help. A very rapid sedimentation rate does not by any means indicate a poor outlook for recovery; it indicates that toxic absorption and tissue destruction are progressing so rapidly that production of biochemical changes in the blood stream is taking place, and this causes the imbalance of the sanguineous elements.

The leucocyte count or filament-nonfilament study in conjunction with the clinical picture is of much greater aid than the sedimentation rate.

Thanks are due Dr. John A. Killian, Director of the Department of Biochemistry, and Dr. J. Carlind Browne, of the Medical Department, of the New York Post-Graduate Medical School and Hospital, for their kind cooperation.

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## LIPASE AND ESTERASE IN THE BLOOD SERUM\*

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### THEIR DIAGNOSTIC VALUE IN PANCREATIC DISEASE

MANDRED W. COMFORT, M.D., AND ARNOLD E. OSTERBERG, PH.D., ROCHESTER, MINN.

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WITH the hope of finding a procedure of greater value than the existing tests in the diagnosis of pancreatic disease, an investigation of the lipase activity of the blood serum was begun one and a half years ago. Rona and Pavlovic, using a stalagmometric method with tributyrin as a substrate, found that serum lipase is inactivated by both quinine and atoxyl, whereas atoxyl inactivates hepatic lipase, and quinine inactivates pancreatic lipase. Methods based on this work had been promising, but had not given uniform results in the hands of various workers and were discarded in favor of one based on the experiments of Cherry and Crandall. These authors demonstrated that an enzyme capable of hydrolyzing olive oil, normally not present, appeared in the blood stream following ligation of the pancreatic ducts of dogs, and that the activity of the enzyme, esterase, normally present in the blood, as measured by hydrolysis of ethyl butyrate or tributyrin, did not show uniform increases. This demonstration indicated that the appearance of an enzyme capable of splitting olive

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\*From the Mayo Clinic.

Submitted for publication, May 5, 1934.

oil in the serum was specific for pancreatic injury, and seemed to offer more logical grounds for the development of a simple, quantitative test.

With these data in mind, the serum of 162 patients has been examined to date, to determine if enzymes capable of splitting olive oil and simple esters are normally present in the serum of man, if the increase of serum lipase activity is specific for pancreatic injury, and if the determination of their activity may be used clinically in the diagnosis of pancreatic disease. Our results encouraged the offering of this preliminary report.

#### METHODS

The serum was obtained from a fasting patient. A modification of the Loevenhart method, used by Cherry and Crandall, with a slightly different buffer solution, was employed. To 1 c.c. of serum were added 3 c.c. of distilled water, 1 c.c. of absolute ethyl butyrate, and 0.5 c.c. of Sorensen's phosphate buffer adjusted to the pH of 7. After shaking thoroughly, the mixture was allowed to stand in the incubator at 40° C. for twenty-four hours, and then was titrated with twentieth normal sodium hydroxide, using 3 drops of 1 per cent phenolphthalein as an indicator, and bringing the solution to the faintest permanent pink. The same method was used in testing the action of lipase, except that 2 c.c. of a 50 per cent emulsion of olive oil in water, emulsified with 5 per cent acaeia (prepared by the Abbott Laboratories) was used, and 0.3 c.c. of 95 per cent alcohol was added to each tube before titrating.

#### RESULTS

*Esterase.*—An enzyme capable of splitting simple esters (esterase) was present constantly in the serum of 162 patients. The fluctuations in its activity bore no constant relation to the condition of the pancreas, and were never marked.

*Lipase.*—An enzyme capable of splitting olive oil (lipase) was present in the serum of 158 of the 162 patients.

The values for lipase activity of patients without known pancreatic disease and of others with known pancreatic disease were studied. In 130 patients without known disease of the pancreas, the range of lipase varied between 0 and 6.4 c.c. twentieth normal sodium hydroxide (per 1 c.c. of serum). In 119 patients, or 92 per cent of the 130, these lipase values fell below 2 c.c., and in 11, or 8 per cent, the values were 2 c.c. or higher. On the other hand, in 32 patients with pancreatic disease, demonstrated at operation or at necropsy, the range of serum lipase activity varied between 0.4 and 10.2 c.c. of twentieth normal sodium hydroxide (per 1 c.c. of serum). In 19, or only 59 per cent, was the lipase activity less than 2 c.c., and in 13, or 41 per cent, the value was 2 c.c. or more (Table I).

The contrast in percentages indicates a relationship between pancreatic disease and serum lipase activity higher than 2 c.c. (for each 1 c.c. of serum).

If the 24 cases in which there was a lipase activity of 2 c.c. or more, in terms of twentieth normal sodium hydroxide (for 1 c.c. of serum), be critically examined for evidence of pancreatitis, activity of such values appears significant

TABLE I  
SUMMARY OF MATERIAL

DIAGNOSIS	LIPASE IN TERMS OF C.C. N/20 NaOH PER 1 C.C. OF SERUM				CASES
	1.9 C.C. OR LESS		2 C.C. OR MORE		
	CASES	PER CENT	CASES	PER CENT	
No organic disease of the liver, biliary tract, or pancreas	54	95	3	5	57
Hepatic disease					
Portal cirrhosis (7 cases)					
Toxic cirrhosis (8 cases)					
Metastatic carcinoma of liver (4 cases)	18	95	1	5	19
Traumatic or malignant stricture of the common bile duct	13	95	1	7	14
Cholecystic disease	7	100	0	0	7
Miscellaneous	6	86	1	14	7
Disease of the common bile duct (without operative discovery of pancreatic disease)	21	81	5	19	26
Total without known pancreatic disease	119	92	11	8	130
Pancreatitis, operative finding	11	55	9	45	20
Carcinoma of head of pancreas	3	66	4	33	12
Total with known pancreatic disease	19	59	13	41	32
Total	138	85	24	15	162

in the diagnosis of pancreatic disease (Table I). Pancreatitis was present in 9 cases, carcinoma of the head of the pancreas in 3 cases, and carcinoma of the ampulla of Vater in one case. In other words, in 13 or 54 per cent of those cases with lipase activity higher than 2 e.c. (for 1 e.c. of serum), pancreatic disease was known to be present (Table I). The explanation of the increased lipase values in the remaining 11 cases (if they are increased and not high normal values) must be sought in disease of the pancreas, intestinal mucosa, liver, or spleen, since Cherry and Crandall have shown that an enzyme capable of splitting olive oil is present in appreciable amounts only in the tissues of these organs. Splenic disease does not occur in these cases. In two cases there was injury caused by obstructive jaundice from carcinoma of the gallbladder, and of toxic hepatitis associated with lobar pneumonia. Hepatic injury might be advanced in explanation of the lipase values. However, this is unlikely, since in thirty-eight other patients with hepatic injury, in many instances much more severe, due to portal and toxic cirrhosis, metastatic carcinoma, obstructive jaundice secondary to benign traumatic stricture, and carcinoma of the gallbladder and ducts, the lipase value was below 2 e.c. (Table I). In fact, it seems as reasonable to suppose that the late postoperative rise in serum lipase values of the patient with carcinoma of the gallbladder was due to pancreatitis which had followed introduction of infection into the biliary passages, and that the high lipase values of the patient with lobar pneumonia and toxic hepatitis were due to associated toxic pancreatitis. In the five cases of stone in the common bile duct in which there were symptoms of cholangitis, pancreatitis is a more reasonable explanation of the elevated serum lipase content than any possible hepatic injury. In the remaining cases (duodenitis, inoperable carcinoma of the stomach, malfunctioning gastroenteric stoma, and peritonitis) although

hepatic injury is hardly a probability, some destruction of intestinal mucosa may have taken place. Although these data do not prove conclusively that an elevated serum lipase value is specific for pancreatic disease, they do show that pancreatic disease accounts for the rise to more than 2 e.c. in serum lipase values in more than 50 per cent of cases in which the value is thus elevated and probably for a much larger percentage. If combined with symptoms of biliary stone or infection, or suggestive of pancreatitis, the finding of lipase activity higher than 2 e.c. was strong confirmatory evidence of pancreatic disease.

If the twelve cases in which the lipase value was 3 e.c. or more are studied in the same way, nine patients, or 75 per cent, are known to have pancreatitis and three, or 25 per cent, may be assumed to have pancreatitis. The reasons for postulating pancreatitis in the cases of carcinoma of the gallbladder, toxic hepatitis due to pneumonia, and peritonitis as the cause of the lipase activity, have been given. In other words, pancreatitis is the most likely explanation in all twelve cases in which the lipase values are high. On further study, a reading of 3 e.c. or more may prove to be almost certain evidence of pancreatic disease.

Carcinoma of the ampulla of Vater and of the head of the pancreas, and acute pancreatitis, are the two diseases of the pancreas so far associated with increased activity of serum lipase. The increase seems to follow both inflammation of the gland and obstruction of the ducts. In 43 per cent of the cases of carcinoma of the head of the pancreas in which examinations have been made so far, the serum lipase readings were higher than 2 e.c. Such a percentage may be greater than might be expected theoretically. Baldwin has shown that the main and accessory pancreatic ducts anastomose at about the level of the neck of the gland, and that both empty separately into the duodenum in more than 80 per cent of cases. When the ducts are so arranged, the tumor, if in the head of the gland, must occlude both ducts in order that obstruction may occur. Tumors of the neck or body of the gland may produce obstruction by occluding the main pancreatic duct before the accessory and main pancreatic ducts anastomose. When the duodenal opening of the accessory pancreatic duct is not patent, involvement of the main duct alone should lead to obstruction. The influence of the last-mentioned anatomic arrangement of the ducts, as well as the elevation of serum lipase activity which follows obstruction, are well illustrated in the following case:

#### REPORT OF CASES

CASE 1.—A married woman, aged forty-eight years, registered at the Mayo Clinic, Sept. 9, 1933, complaining of abdominal pain, jaundice, and loss of weight. In February, 1932, she had had diffuse, cramping pain in the abdomen that had lasted a few hours; this had been followed by a slight chill, the development of jaundice, and passage of clay-colored stools and dark urine. These symptoms had cleared within four days; two weeks later a similar episode had occurred. In October, 1932, four severe abdominal cramps, each lasting a half day, had occurred. These had been followed by severe chills, fever, and jaundice. The jaundice had fluctuated, but had remained until the end of December, 1932. During this time the stools had varied from black to clay color, and the patient had lost considerable weight and strength. Severe pruritus also had been noted.

The patient was emaciated and markedly jaundiced. The abdomen was moderately distended. Resistance to palpation in the right upper quadrant of the abdomen, and questionable ascites, were noted. The temperature was 103° F. and the pulse rate 120 beats per minute. The temperature fluctuated irregularly, varying from 99° to 103° F. Urinalysis repeatedly gave negative results, except for the presence of bile and occasional granular casts and pus cells. Serologic tests for syphilis gave negative results. The concentration of hemoglobin was 48 per cent (Dare); erythrocytes numbered 2,600,000, and leucocytes 17,800 per cubic millimeter of blood. Of the leucocytes 92.5 per cent were polymorphonuclears. The concentration of blood sugar was 83 mg. for each 100 c.c. of blood. Determinations of serum bilirubin and serum lipase may be noted in Fig. 1. The stools repeatedly gave negative results for bile, and intermittently gave positive results for blood. The test

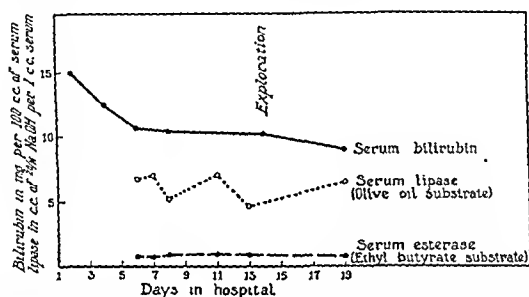


Fig. 1.—(Case 1.) The increased concentration of serum bilirubin and the serum lipase activity in obstruction of the pancreatic and common bile ducts caused by carcinoma of the ampulla of Vater. The esterase activity is not increased.



Fig. 2.—(Case 1.) Specimen obtained at necropsy; dilatation of the main and accessory pancreatic ducts secondary to obstruction by carcinoma of the ampulla of Vater may be noted.

for galactose tolerance was negative. The diagnosis was stone in the common bile duct, obstructive jaundice, and cholangitis.

After the patient had been given solution of glucose and of calcium chloride intravenously, and a transfusion of blood, operation was performed September 22. Approximately 800 to 1,000 c.c. of turbid fluid escaped when the abdomen was opened. The liver contained multiple small carcinomatous masses. The gallbladder was distended, but its walls were normal in color and thickness. An indurated mass, approximately 5 cm. in diameter, at the head of the pancreas was found; this seemed to be carcinoma. The wound was closed after exploration. The postoperative course was not at all satisfactory, and the patient died on the sixth day after operation. The concentrations of serum bilirubin and serum lipase remained at a constant level following operation.

At necropsy the liver was somewhat enlarged, weighed 1,700 gm., and contained multiple carcinomatous masses. The gallbladder was dilated and contained approximately twenty stones; its walls appeared normal. The intrahepatic and extrahepatic bile ducts were markedly dilated; four small stones were found in the common bile duct, but were not large enough to obstruct the dilated duct. The pancreatic ducts were likewise dilated (Fig. 2), and the accessory duct ended in a blind pouch behind the common bile duct. The orifice of the ampulla was almost completely occluded by a polypoid mass measuring 1.5 by 1 by 1 cm. in diameter, which on microscopic examination proved to be a carcinoma. Metastasis had involved the regional lymph nodes. Microscopic examination of the pancreas revealed no evidence of infection.

*Comment.*—The obstruction and marked dilatation of the pancreatic duct (Fig. 2) was possible only because the accessory duct ended in a blind pouch and did not open into the duodenum. In dogs, complete obstruction is followed by prompt initial rise and subsequent disappearance of lipase from the serum. A secondary rise may occur. Since complete obstruction is not associated experimentally with prolonged elevation of the lipase values, the incompleteness of the obstruction probably accounts for the continued secretion and absorption of the pancreatic juice, and for the duration of the increased values in the blood stream.

Although little evidence of cholangitis was found at necropsy, cholangitis, as well as stones in the common bile duct, may possibly have played some part in causing the symptoms. Although clinically the picture simulated closely that of stone in the common bile duct, obstructive jaundice, and cholangitis, the widespread metastasis that arose from a small carcinoma of the ampulla of Vater is indisputable evidence that the carcinoma had been present much longer than the last episode of jaundice, which had persisted for three months.

In 45 per cent of the cases of pancreatitis, in which the condition had been found at operation or at necropsy, the lipase values were higher than 2 c.e. of sodium hydroxide (per 1 c.e. of serum). In no instance was chronic disease associated with serum lipase values higher than 2 c.e. of serum. In only one instance did acute disease of the pancreas fail to produce activity higher than 2 c.e. In this case the disease was of a fulminating type, with almost complete destruction of the organ. Destruction of the finer blood and lymph vessels may have prevented absorption, and may explain the values obtained in this case. The elevation of the lipase values in association with degrees of pancreatitis not quite great enough to be more than suspected clinically, as in Case 2, is a more important observation.

CASE 2.—A married woman, aged thirty-five years, registered at the clinic Nov. 9, 1933, complaining of attacks of abdominal pain and jaundice. In the last six years she had had recurrent attacks of colicky pain in the epigastrium, that had extended to the back, had lasted two or three days, and had occurred three or four times yearly. Between attacks there had been intolerance to cabbage, beans, and greasy food, with considerable gaseous dyspepsia and nausea. In the last two months the attacks had been more frequent and severe. October 24, a sudden, severe epigastric pain had occurred, which had extended to the right shoulder, had recurred for hours, had required morphine for relief, and had been associated with soreness in the right upper quadrant of the abdomen. October 28, jaundice had appeared, the stools had become dark and the urine light, and generalized pruritus had developed. Occasional, colicky pains had recurred, and the jaundice had fluctuated in degree.



At examination the jaundice was marked. Tenderness deep in the right upper quadrant could be elicited by pressure. A mass near the median line, thought to be the distended gallbladder, could be felt. Urinalysis repeatedly gave negative results, except for bile and granular casts. Examination of the blood and serologic tests for syphilis gave negative results. November 12, another colic occurred, followed by elevation of temperature to 101° F. and increase in the concentration of bilirubin from 10.2 to 16.2 mg. per 100 c.c. of serum. The lipase value was 10.2 c.e. (Fig. 3). The diagnosis was chronic, subacute cholecystitis with stones, stone in the common bile duct, obstructive jaundice, and pancreatitis.

Exploration, carried out two days later, revealed that the gallbladder was subacutely inflamed; its walls were thickened and edematous. Three stones, varying in size from 2.5 cm. to 3 cm., were removed from it. The head of the pancreas was enlarged and brawny, with diffuse inflammation of marked subacute pancreatitis. The common bile duct was opened and examined but no stones were found; in its walls was the same degree of inflammation as in the gallbladder. Whitish, puslike material was present in the bile. The scoop was passed into the duodenum easily. A T-tube was placed in the common bile duct. The liver had changed to almost greenish brown, and its lobules stood out as punctate spots.

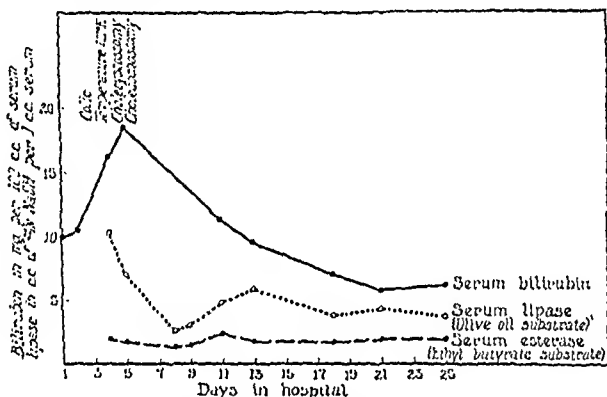


Fig. 3.—(Case 2.) The increased concentration of bilirubin and the serum lipase activity in cholangitis, hepatitis, and pancreatitis. The serum lipase activity at its maximal during an attack of pain, chill, and fever, declined in a manner parallel to the concentration of serum bilirubin with the subsidence of the acuteness of the process. The esterase activity is not increased.

The postoperative course was satisfactory, with the exception of an elevation of temperature on the twelfth and thirteenth days.

*Comment.*—The rise in the lipase values following the attack of obstruction of the common bile duct and pancreatitis, as well as the fall in lipase values postoperatively (Fig. 3), well illustrate the influence of an acute process and its subsequent resolution on the serum lipase activity. The clinical symptoms did not point to pancreatitis. The preoperative diagnosis of pancreatitis depended entirely on the high activity of lipase in the serum. It is possible that the greatest sphere of usefulness of this lipase test of pancreatic function will be found in such cases, and in those in which mild attacks of epigastric distress occur without definite clinical, roentgenologic, or other laboratory evidence of cholecystic or pancreatic disease.

In neither of these two cases in which disturbance of the enzymatic function took place was there alteration in the islands of Langerhans sufficient to produce glycosuria or hyperglycemia. In only two cases of pancreatitis in the

series did such glycosuria appear. The lipase activity of the serum promises to be a more sensitive index of pancreatic dysfunction than glycosuria or hyperglycemia.

#### SUMMARY AND CONCLUSIONS

An enzyme capable of splitting simple esters (esterase) is normally present in the blood serum, but its activity bears no consistent relationship to any disease so far observed by us. An enzyme capable of splitting olive oil (lipase) usually is present in the serum of man. In 80 per cent of the cases the lipase activity in terms of twentieth normal sodium hydroxide is less than 2 c.e. (per 1 c.e. of serum). Although a greater number of patients without disease must be examined before a final statement can be made regarding the range of normal value for lipase activity, it appears that the upper limit is in the region of 2 c.e. (per 1 c.e. of serum). The activity of serum lipase was higher than 2 c.e. of twentieth normal sodium hydroxide (per 1 c.e. of serum) in 45 per cent of the cases of pancreatitis, and in 33 per cent of the cases of carcinoma of the head of the pancreas and of the ampulla of Vater. Such values were due to acute or subacute pancreatitis rather than to chronic pancreatitis, or to obstruction to the outflow of the pancreatic juice, due to carcinoma of the head of the pancreas. Lipase activity higher than 2 c.e. (per 1 c.e. of serum) was also encountered in eleven (6 per cent of the 162 patients) who had duodenitis, inoperable carcinoma of the stomach, carcinoma of the gallbladder, toxic hepatitis secondary to lobar pneumonia, or stone in the common bile duct with cholangitis or peritonitis. It has been pointed out, however, that high values may not primarily be related to such diseases, but to associated pancreatic disease. Although further experience with increased serum lipase activity as a test of disturbed function of the pancreas is needed to establish the specificity of its relationship to that organ, the test appears at present to be good evidence of disturbed pancreatic function, especially when associated with the signs and symptoms of cholecystitis, choledocholithiasis, cholangitis, and pancreatitis.

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# THE PROGNOSIS OF CORONARY THROMBOSIS BASED ON THE NONPROTEIN NITROGEN IN THE BLOOD\*

CHARLES LEROY STEINBERG, M.D., ROCHESTER, N. Y.

NELLIS FOSTER<sup>1</sup> reported eight cases of heart disease in which the non-protein nitrogen was elevated. Four cases of this group came to autopsy; and none showed evidence of nephritis. He suggested that an increase in the nonprotein nitrogen may be a resultant of purely circulatory disturbances. The cases reported by Foster and their respective filtrate nitrogen values are tabulated below.

ANATOMIC DIAGNOSIS	NONPROTEIN NITROGEN
(1) Cardiac, valvular	40 mg.
(2) Cardiac, mitral stenosis	59 mg.
(3) Cardiac, aortic insufficiency	61 mg.
(4) Cardiac, aortic insufficiency	47 mg.
(5) Cardiac, mitral stenosis (cerebral emb.)	53 mg.
(6) Cardiac, mitral and tricuspid	73 mg. and 85 mg.
(7) Cardiac, mitral and tricuspid	90 mg.
(8) Cardiac, aortic	43 mg.

He opined, "if these results can be confirmed by other students of these diseases the fact involved is important as indicating the part played by the circulation in a picture already recognized as composite."

In 1933 the author<sup>2</sup> reported sixteen cases of coronary thrombosis in which particular attention was paid to the nonprotein nitrogen. Ten of these reported cases showed a filtrate nitrogen of 40 mg. per 100 c.e. of blood or more. None of these cases showed evidence of a marked nephritis. It was also shown that even a marked drop in blood pressure and a diminution in urinary output were not sufficient in themselves to account for the nonprotein nitrogen rise in coronary thrombosis. During the past three years, the author has been able to collect thirty-one cases of heart disease due to coronary thrombosis in which particular attention has been paid to the nonprotein nitrogen value as regards its relationship to this interesting and more recently diagnosed heart disease.

In Table I, the author attempts to show in cross sectional study the relationship of nonprotein nitrogen, available kidney function test or urinalysis, and the time in which the coronary occlusion may have occurred. A cursory examination of this table will show that, whereas, in some cases the "pthalein" excretion and the nonprotein nitrogen value are in harmony, in other cases there are no such relationships. Still more important is the fact that the kidney shows ability to concentrate well in some cases in which the "pthalein" excretion is diminished. Sufficient cases are included in Table II to indicate that a drop

\*From the Department of Medicine, Rochester General Hospital.  
Received for publication, May 11, 1934.

TABLE I\*

NAME	AGE	DATES OF AT- TACK OR ATTACKS	NONPROTEIN NITROGEN (MG. PER 100 C.C.)	KIDNEY FUNCTION TESTS	URINE
J.S.	59	9/28/33	40—10/ 2/33 31—10/ 5/33 38—10/10/33 27—10/13/33	†PSP—65% 10/4/33	10/2/33 Sp. G. 1020. Neg. alb. Occ. gran- ular cast. 10/10/33 Sp. G. 1030. Occ. R.B.C.
M.B.	43	Minor anginal at- tacks for 2 yr. Typical attack 10/16/33	42—10/18/33 23—10/24/33 40—11/ 9/33 34— 1/13/34 34— 1/26/34 35— 2/ 2/34	PSP—45% 11/21/33	Urine neg. on many occasions. Sp. G. 1030 on 11/19/33. Tr. alb.
E.C.M.	52	Minor anginal at- tacks since Dec., 1930	44—12/ 3/31 50— 3/12/32 48— 3/14/32 50— 3/21/32 47— 6/ 7/32	PSP—52% 12/7/31 PSP—65% 3/19/32 PSP—30% 6/16/32 3/22/32 Sp. G. 1023 to 1027	10/7/31—Sp. G. 1022. Tr. alb.; occ. granu- lar and hyaline cast. 3/13/32—Sp. G. 1023. Tr. alb.
R.S.	67	Minor anginal at- tacks for past 4 to 5 yr. Severe attack 10/17/33	36—10/20/33 41—10/23/33 38—10/26/33 32—10/31/33	PSP—40% 10/21/33	10/17/33 negative. 10/31/33 negative. 11/11/33—Sp. G. 1020; tr. alb., occ. R.B.C. Rare hyaline cast.
G.C.	47	Severe attack Jan., 1932, and May 5, 1933	150— 9/12/33	PSP—80% 2/22/30	9/12/33—Sp. G. 1020; tr. alb.
L.P.	42	Severe typical at- tack first and third weeks July, 1933	40— 7/26/33 30— 8/12/33 31— 8/19/33	PSP—50% 8/12/33	7/26/33—Sp. G. 1028; 3 R.B.C./HPF. Occ. epithelial and gran. cast.
A.M.	59	First attack June, 1930. Second attack Aug., 1933	33— 2/18/34 43— 2/20/34 37— 2/23/34 34— 2/27/34 40— 3/ 2/34 50— 3/ 6/34 52— 3/13/34	None	3/14/34—Sp. G. 1017. Two-plus alb. Neg. microscopic.
F.C.	30	First attack May, 1930; several minor attacks Aug. to Oct., 1930	33— 7/17/31 34—12/23/31 33—12/28/31	None	7/17/31 negative 10/25/31 negative 12/24/31 negative 5/ 8/32 negative
N.D.	51	Attack 5/22/33	23— 5/29/33 20— 6/ 5/33 19— 6/16/33	PSP—42% 6/22/33	Negative on several oc- casions
W.G.	49	Typical attacks 6/14/33 and 6/16/33	21— 6/17/33 32— 6/19/33 35— 6/22/33 30— 6/26/33 33— 6/29/33	PSP—33% 6/19/33 PSP—59% 6/29/33	6/18/33—Sp. G. 1020; Neg. except for few hyal. and gran. casts.
J.M.D.	65	Attacks August, 1932, and Aug- ust, 1933	37— 8/22/33 35— 8/26/33 34— 8/29/33	None	8/25/33—Sp. G. 1023; 40 to 50 R.B.C./HPF.

\*This table includes all cases that were not reported in entirety or at all in the author's preliminary report.

†This test in this paper denotes the two-hour intravenous method.

TABLE I—CONT'D

NAME	AGE	DATES OF AT- TACK OR ATTACKS	NONPROTEIN NITROGEN (MG. PER 100 C.C.)	KIDNEY FUNCTION TESTS	URINE
M.M.M.	65	Minor anginal at- tacks for three years; severe attack 1/23/32	24— 1/25/32	None	1/25/32—Sp. G. 1025; V. Ft. Tr. albumin
M.M.	51	Severe typical at- tack 2/6/32; minor anginal attacks since	43— 2/15/32 39— 3/23/32	PSP—60% 2/15/32; Mosenthol 2/21/32 Sp. G. 1010 to 1025	2/14/32—Sp. G. 1012 negative
W.T.C.	71	Typical attack 4/21/33	44— 4/11/33 67— 4/22/33 34— 4/24/33 36— 4/28/33 28— 5/ 2/33 28— 5/12/33 24— 5/23/33	PSP—35% 4/26/33 PSP—70% 5/5/33	Frequent urinalyses negative
C.B.	58	Typical attack 1/14/33	39— 1/16/33 34— 1/23/33 35— 1/25/33 32— 1/27/33 200— 1/30/33	PSP—65% 1/23/33	1/2/33—Negative ex- cept for one-plus albumin

TABLE II

CASE	BLOOD PRESSURE (DATES)		NONPROTEIN NITROGEN	INTAKE	OUTPUT
J.M.D.	8/20/33	140/100		700 c.c.	40 c.c. (Aur. fibrillation)
	8/21/33	120/ 80		Not recorded	(Aur. fibrillation)
	8/22/33	70/ 60	37	1,280 c.c.	550 c.c. (Aur. fibrillation)
	8/23/33	100/ 70		Not recorded	(Aur. fibrillation)
	8/24/33	100/ 80		1,640 c.c.	1,200 c.c. Regular rhythm
	8/26/33	100/ 60	35	1,360 c.c.	750 c.c. Regular rhythm
	8/28/33	Not obtained		1,240 c.c.	500 c.c. Regular rhythm
	8/29/33	Not obtained		Incontinent	
	8/30/33	Not obtained		Incontinent	
W.G.	6/16/33	100/?		Suppression for 24 hr.	(Aur. fibrillation)
	6/17/33	94/ 80	21	1,460 c.c.	1,200 c.c. (Aur. fibrillation)
	6/18/33	100/ 80		2,080 c.c.	800 c.c. (Aur. fibrillation)
	6/19/33	100/ 76	32	2,830 c.c.	500 c.c. (Aur. fibrillation)
	6/20/33	80/ 60		1,440 c.c.	1,000 c.c. (Aur. fibrillation)
	6/21/33	94/ 70		2,400 c.c.	980 c.c. (Aur. fibrillation)
	6/22/33	94/ 64	35	1,400 c.c.	800 c.c. (Aur. fibrillation)
	6/23/33	100/ 60		2,440 c.c.	1,100 c.c. (Aur. fibrillation)
	6/25/33	100/ 70	30 (6/26)	1,640 c.c.	850 c.c. (Aur. fibrillation)
	6/28/33	102/ 60			
	6/29/33		33		
C.B.	1/14/33	140/ 90 (9:00 A.M.)			
	1/14/33	110/ 80 (7:20 P.M.)			
	1/15/33	100/ 80		2,490 c.c.	500 c.c.
	1/16/33	88/ 65	39	2,950 c.c.	400 c.c. (Aur. fibrillation)
	1/19/33	85/ 60		1,100 c.c.	650 c.c. Normal rhythm
	1/20/33	75/ 55	35	2,300 c.c.	1,100 c.c. (Quinidine)
	1/21/33	85/ 60		1,830 c.c.	880 c.c.
	1/23/33	70/ 45	34	2,150 c.c.	600 c.c.

This table indicates that a drop in blood pressure and a diminution in urinary output may not influence the filtrate nitrogen in acute coronary occlusion. Adson and Brown<sup>6</sup> have found that a drop in blood pressure in essential hypertension may not influence the vital function of the kidneys.

in blood pressure and a diminution in urinary output may not increase the nonprotein nitrogen in coronary thrombosis. In the remaining three tables (Tables III, IV, and V), complete follow-up studies on the thirty-one cases are given. (The author wishes to apologize for repetition from his preliminary report, but this was necessary in order to give a more complete and vivid picture of the value of repeated nonprotein nitrogen studies in this disease.)

A careful review of the latter three tables reveals that a rise in the nonprotein nitrogen value is very common in acute coronary occlusion, that an elevated value in the filtrate nitrogen (particularly if it remains elevated in serial studies) offers a very, very poor prognosis, and that a normal nonprotein nitrogen or one that recedes from a higher to a normal value is of better prognosis. Continued studies may not show such optimistic conclusions as to the prognostic value of repeated nonprotein nitrogen studies in this heart disease, but this second paper should offer a stimulus for further study in this field.

*Electrocardiography.*—Forty-one electrocardiographic tracings were made on twenty-eight cases of the total thirty-one. The following abnormalities were noted: (A) shurring or notching of the QRS complex in twenty-one tracings;

TABLE III

TABULAR STUDY OF CASES SHOWING A NONPROTEIN NITROGEN BELOW 40 MG. PER 100 C.C. BLOOD

CASE	NONPROTEIN NITROGEN (MG. PER 100 C.C.)	OUTCOME	DATE (LAST CHECK-UP)
A.G.	30— 8/17/31	Alive	Dec. 26, 1933
M.Me.	33— 9/10/31	Alive	Dec. 20, 1933
A.P.	34—12/10/31	Alive	Dec. 20, 1933
F.C.	33— 7/17/31 34—12/23/31 33—12/28/31	Alive	Dec., 1933
N.D.	23— 5/29/33 20— 6/ 5/33 19— 6/16/33	Alive	Dec. 20, 1933
W.G.	21— 6/17/33 32— 6/19/33 35— 6/22/33 30— 6/26/33 33— 6/29/33	Alive	Nov., 1933
M.M.M.	24— 1/25/32	Died	July, 1932 (lived for 6 mo after occlusion)
S.L.	32— 1/26/31	Died	1/27/31
J.M.D.	37— 8/22/33 35— 8/26/33 34— 8/29/33	Died	Aug. 30, 1933
G.H.	37— 4/17/31	Died	April 25, 1931
C.B.	34— 1/ 8/34 31— 1/13/34 30— 1/16/34 38— 1/19/34 33— 1/26/34 30— 1/30/34 33— 2/ 2/34 33— 2/ 6/34	Alive	Feb. 12, 1934

TABLE IV

TABULAR STUDY OF CASES SHOWING AN ELEVATED NONPROTEIN NITROGEN

CASE	NONPROTEIN NITROGEN (MG. PER 100 C.C.)	OUTCOME	DATE
J.B.	48— 3/28/32	Died	May, 1932
S.A.	46— 1/ 2/31	Died	Feb. 6, 1931
C.P.	71— 8/ 3/31	Died	Aug. 14, 1931
	95— 8/ 7/31		
	133— 8/10/31		
	185— 8/11/31		
	215— 8/14/31		
J.T.	42—11/24/31	Died	Soon after leaving hospital, spring, 1931
B.W.	41— 3/ 6/32	Died	4/1/32
	40— 3/ 9/32		
	142— 3/28/32		
A.M.B.	60— 4/30/32	Died	July, 1932
	47— 5/ 3/32		
	40— 5/10/32		
	54— 6/10/32		
L.M.	40— 8/ 9/32	Died	Aug. 14, 1932
C.B.	39— 1/16/33	Died	Jan. 30, 1933
	35— 1/20/33		
	34— 1/23/33		
	35— 1/25/33		
	32— 1/27/33		
	200— 1/30/33		
E.C.M.	44—12/ 3/31	Died	June 22, 1932
	50— 3/12/32		
	48— 3/14/32		
	50— 3/21/32		
	47— 6/ 7/32		
G.C.	150— 9/12/33	Died	Sept. 14, 1933
M.M.	43— 2/15/32	Died	Spring, 1932
	39— 3/23/32		
A.M.	33— 2/18/34	Condition serious date of writing	
	43— 2/20/34		
	37— 2/23/34		
	34— 2/27/34		
	40— 3/ 2/34		
	50— 3/ 6/34		
	52— 3/13/34		

(B) ventricular premature contractions in six tracings; (C) auricular premature contractions in one case; (D) auricular fibrillation in seven tracings; (E) high take-off of the T-wave in three tracings; (F) an inverted T-wave in Lead I or II (or both) in thirteen tracings; (G) an upper convexity of the ST-interval in six tracings; (H) a deep  $Q_s$  in five tracings; (I) left ventricular preponderance in eighteen tracings; (J) right ventricular preponderance in one case; (K) a delayed auricular-ventricular conduction time in one case; (L) and nodal rhythm in one case. In other words, there was evidence of coronary disease in twelve of the forty-one tracings. The history of viselike or crushing pain in the region of the precordium radiating down the left arm or up the left side of the neck plus a drop in blood pressure seems to be most common diagnostic criterion of coronary thrombosis. Only occasionally is the

TABLE V  
TABULAR STUDY OF CASES SHOWING A REGRESSION OF NONPROTEIN NITROGEN

CASE	NONPROTEIN NITROGEN (MG. PER 100 C.C.)	OUTCOME	DATE (LAST CHECK-UP)
P.D.	75—2/28/31 30—3/16/31	Alive	Dec. 20, 1933
H.DeW.	110—11/27/31 42—11/30/31 38—12/4/31	Alive	Oct., 1933
S.S.	29—10/18/32 45—11/5/32 27—11/14/32	Alive	Dec., 1933
J.S.	40—10/2/33 31—10/5/33 38—10/10/33 27—10/13/33	Alive	Jan. 1, 1934
R.S.	36—10/20/33 41—10/23/33 38—10/26/33 32—10/31/33 35—11/3/33 32—11/8/33	Alive	Jan. 21, 1934
L.P.	40—7/26/33 30—8/12/33 31—8/19/33	Alive	Sept. 5, 1933
W.T.C.	44—4/11/33 67—4/22/33 34—4/24/33 34—4/26/33 36—4/28/33 28—5/2/33 27—5/5/33 28—5/12/33 24—5/16/33 24—5/23/33	Alive	Dec., 1933
M.B.	42—10/18/33 23—10/24/33 40—11/9/33 39—11/15/33 39—1/8/34 34—1/13/34 34—1/16/34 34—1/19/34 34—1/26/34 35—2/2/34	Alive	Feb. 12, 1934

electrocardiogram of value in the definite diagnosis of coronary thrombosis. At no time is it an emergency diagnostic procedure.

*Serology.*—The complement fixation test was negative in twenty-eight cases. The Kahn precipitation test was negative in the same twenty-eight patients. The serology was positive in the remaining three cases. The total percentage of syphilitic cases in this group was, therefore, 9.6 per cent. Syphilis, meaning positive Wassermann reactions, was noted in 4.5 per cent of Levine and Brown's cases,<sup>3</sup> and in 14 per cent of Conner and Holt's cases.<sup>4</sup> Warthin<sup>5</sup> believed that syphilis was a predisposing factor to coronary disease.

*Comment.*—Nellis Foster was probably the first observer to note that uremia might be associated with circulatory disturbances, and that the uremia may occur in such cases without nephritis. In his cases, he did not include coronary



occlusion. The author probably was the first observer to note that a high nonprotein nitrogen in the blood is very often associated with coronary thrombosis. In fact, a filtrate nitrogen value of 40 mg. or above was noted in twenty of the thirty-one cases reported in this paper.

#### SUMMARY

Thirty-one cases of heart disease due to coronary thrombosis are presented. The clinical history in all these cases was typical. No atypical cases are included in this study. (1) Uremia seems to be a common finding in coronary thrombosis. (2) Serial studies of the nonprotein nitrogen in the blood may offer an aid in prognosis. (3) A filtrate nitrogen that remains high or continues to rise is of ill omen, whereas, a lower nonprotein nitrogen or one that recedes is of much better prognosis. (4) Further studies in this interesting field are indicated.

The author wishes to express his gratitude to Dr. David A. Haller, Chief of the Medical Service, who has made this study possible. He thanks Dr. M. E. Missal for the complete record of case G. C.

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# LABORATORY METHODS

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## A MODIFIED TECHNIC FOR STERNAL PUNCTURE AND ITS VALUE IN HEMATOLOGIC DIAGNOSIS\*

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CARL REICH, A.B., M.D., NEW YORK, N. Y.

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THE bone marrow is the largest organ in the body and comprises 4.6 per cent of the body weight.<sup>1</sup> It is the site of production of the erythrocytes, leucocytes, and platelets, and the cellular content of the peripheral blood reflects the changes going on in the parent tissue. In the past the study of blood dyscrasias was aided by sternal biopsy with the trephine. This procedure has to be done in the operating room, however, and is sometimes quite an ordeal for a sick patient. In addition, it cannot be repeated at frequent intervals, as is the case with sternal puncture.

Arinkin<sup>2</sup> was the first to use sternal puncture to determine the cellular composition of the marrow. Shortly after, Arjeff<sup>3</sup> devised a special needle to facilitate this technic. A detailed description of our method follows:

The site selected for puncture is in the middle of the sternum just below the juncture of the body with the second rib. A small spot 1 cm. in diameter is painted with iodine. The skin over this point is infiltrated with 1 per cent

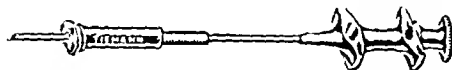


Fig. 1.

novocaine, and the novocaine is then injected straight down to the sternum and the periosteum is infiltrated. This step is important to make the puncture painless. The marrow needle is similar in construction to that of Arjeff, except that its bore is 10 gauge and the guard can be lowered to within 0.2 cm. of the tip to make it available for children also. For adults the guard is set at 1 cm. from the point of the needle. To make the puncture, the sterile needle is pushed vertically through the spot prepared as above until it reaches the periosteum. If the outer plate of the sternum is not too thick, the needle can be pushed through by hand. If the resistance is too great a few gentle taps with a small mallet will suffice to drive the needle into the spongy bone. The stylet is removed and a sterile 20 c.c. record transfusion syringe is attached and suction applied. If the needle is deep enough bloody fluid will appear in the syringe. If not, the stylet is replaced, the guard raised a few turns, and the needle is driven in until blood can be sucked out. About 10 c.c. of the bloody fluid is

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\*From the Lenox Hill Hospital, and City Hospital.  
Received for publication, February 24, 1934.

aspirated and mixed in a 15 c.c. centrifuge tube with 2 c.c. of a 1.4 per cent sodium oxalate solution. This has been shown to be an isotonic anticoagulant which does not change the volume of the cells.<sup>4</sup> The mixture is now centrifuged about five minutes at moderate speed. The supernatant plasma is removed, using a capillary pipette with a rubber nipple attached. By carefully manipulating the same pipette, the buffy coat is removed and transferred to a tube made by sealing off one end of a short piece of glass tubing of slightly larger diameter than the pipette. This is again centrifuged for five minutes and after the plasma is removed the buffy coat is pipetted off and smears are made on slides and stained as follows: Jenner stain, three minutes; distilled water, three minutes; decant; flood with dilute Giemsa, 1 drop to 1 c.c., thirty minutes; wash and dry. A differential count of 1,000 cells is then made.

The aforementioned authors<sup>2, 3</sup> made their smears directly from the aspirated sternal contents and a count of 1,000 cells was extremely tedious. The technique of concentrating the cells, as described here, has already been found of value in studying the peripheral blood.<sup>5</sup> Without distorting the cells, or interfering with their staining properties, this method permits a rapid survey of the sternal contents with an easy count of 1,000 cells. The normal differential count is approximately as follows:

Polymorphonuclear neutrophiles (mature)	25%
Polymorphonuclear neutrophiles (young forms)	5%
Polymorphonuclear neutrophiles (band forms)	10%
Lymphocytes	10%
Monoocytes	1%
Eosinophiles	1%
Myeloblasts	2%
Premyelocytes and myelocytes	20%
Erythroblasts	23%
Proerythroblasts	2%
Plasma cells	1%

A few actual cases may be of value in illustrating the practical importance of this procedure:

CASE 1.—The patient was a white female, fifty-five years old. She was well up to three months before admission to the hospital, when she began to lose strength and complained of shortness of breath. On admission to the hospital, she looked acutely ill and had a temperature of 101°. She was very pale, the spleen was moderately enlarged and petechial hemorrhages were present in the mouth and over the skin. Her blood count was hemoglobin 40 per cent, R.B.C. 2.5 mil., W.B.C. 128,000. Differential count, neutrophiles 2 per cent, lymphocytes 8 per cent, myelocytes 1 per cent, myeloblasts ? 89 per cent. There was some doubt if these immature cells were myeloblasts. Marrow puncture showed erythroblasts 5 per cent, myeloblasts 95 per cent. The diagnosis was therefore definitely established as myeloblastic leucemia.

CASE 2.—The patient was a white female, seventy years old. For the past six months she had been losing strength and had become markedly anemic. She had also lost her appetite and had no desire for food. The tongue was somewhat atrophic and the skin lemon yellow in color. Gastric analysis revealed no free HCl. The blood count was hemoglobin 20 per cent, R.B.C. 1.0 mil., W.B.C. 9,800. Differential count, neutrophiles 63 per cent, lymphocytes 20 per cent, monoocytes 15 per cent, eosinophiles 1 per cent, basophiles 1 per cent. The differential diagnosis was between pernicious anemia and gastric malignancy. Owing

to her weakened condition G.I. series was deferred. Bone marrow revealed, neutrophile polys (mature) 36 per cent, neutrophile polys (young forms) 3 per cent, neutrophile polys (band forms) 13 per cent, eosinophiles 1 per cent, lymphocytes 22 per cent, myeloblasts 2 per cent, premyelocytes and myelocytes 20 per cent, erythroblasts 1 per cent, plasma cells 2 per cent. A bone marrow showing only 1 per cent erythroblasts could not be that of a pernicious anemia. This fact was completely substantiated when x-ray revealed a carcinoma of the stomach.

CASE 3.—The patient was a white female, forty years old. The history was of three weeks' duration and the patient complained of marked prostration and of a severe ulceration of the mouth. The temperature was 100.5°. The blood count was hemoglobin 80 per cent, R.B.C. 4.4 mil., W.B.C. 1,000. Differential count, neutrophiles 23 per cent, lymphocytes 73 per cent, monocytes 4 per cent. The diagnosis was agranulocytic angina with a possibility of a leucopenic lymphatic leucemia. Sternal puncture showed neutrophile polys (mature) 22 per cent, neutrophile polys (young) 6 per cent, neutrophile polys (band forms) 16 per cent, lymphocytes 10 per cent, myeloblasts 2 per cent, myelocytes and premyelocytes 21 per cent, erythroblasts 21 per cent, proerythroblasts 1 per cent, plasma cells 1 per cent. In this case a study of the marrow by sternal puncture not only definitely ruled out a lymphatic leucemia, but showed that this was a case of agranulocytosis with maturation arrest, rather than marrow aplasia.

It is therefore seen that the above described method of sternal puncture is simple, readily adaptable for routine hospital use, and is of real value in getting additional information in the study of the blood dyscrasias.

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## COMMENTS AND PROCEDURE ON THICK BLOOD FILM TECHNIC\*

JOAQUIN BENAVIDES, L. T., PANAMA, R. DE P.

THE advent of the thick-film method for the examination of dried blood films that combined laking and staining in one step was a fine advance in laboratory diagnosis. Barber and Komp's<sup>1, 2, 3</sup> modifications to improve the quality of the preparations and simplify the mechanical handling of the slides for mass rural survey work was another distinct advance in technic. This is an excellent time-saving method for community work and can be applied equally well to surveys of domestic and wild animals if confined to mammalian life.<sup>2, 3</sup> The benefits derived from the relatively new method certainly invite a much wider use than at present prevails among field, clinical and research workers. This is particularly true for those who are located in tropical regions. It permits the use of four or five times as much blood as can be made into a satisfactory thin film and reduces the searching time from about thirty minutes to three or five minutes. A vast number of large parasites such as microfilaria, trypanosomes and even the large forms of malaria parasites are carried away by the instrument used in spreading a thin film. None of the blood taken by the thick-film method need be lost by the use of a spreading or stirring instrument. Cycles in the chronic stage of a disease can be more easily followed. It is even helpful in preliminary diagnostic work in revealing the presence of leucemia, leucopenia, leucocytosis, eosinophilia and polychromatophilia. The writer recently found a case of myelogenous leucemia during the course of a survey for malaria. This man had not been under treatment and was not aware that he had a condition that warranted attention.

An experienced technician can examine with a fair degree of accuracy 125 thick films in seven hours provided that the films are well prepared and do not exceed a half inch in diameter. The writer's experience covers twelve years in the tropics and four years of that time have been very largely spent in thick, blood film work. This personal experience with the method is not given with the intention of matching it with others who may already employ the method but rather to stimulate fellow technicians in its use and to mention the latitude the technic permits in its application.

*Slides.*—One should be sure that all slides fit the slide boxes. Frequently, they are too long or too short or even too thick. Very thin slides offer trouble with the use of the mechanical stage. We use green tinted slides rather than the colorless or clear slides. They are capable of longer use and stand cleaning better.

\*From the Gorgas Memorial Laboratory.

Received for publication, February 20, 1931.

New slides should be cleaned and soaked in alcohol before use, and it is very necessary to have them perfectly dry and free of dust as well as fingerprint grease.

Used survey slides should have the cedar oil and stained blood film removed as far as possible by rubbing them with gauze moistened with xylol before they are immersed in a hot (not boiling) solution of a mild soap. The slides are then washed individually in running water and then immersed in 70 per cent alcohol overnight. They should be dried with a clean gauze cloth and placed in the standard cardboard shipping boxes lined with gauze. Dust from the cardboard will collect on them after long travel in boats, cars, or on mules unless this measure is taken.

*Equipment.*—The equipment required depends on whether it is intended for hospital or field work. None is needed for hospital work except that an extra slide should be taken on which to make the film. One large drop of

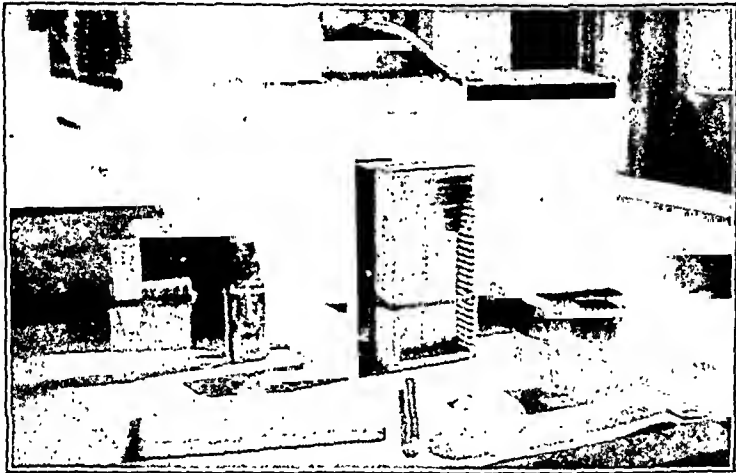


Fig. 1.—A view of the slide box and its stand, the field case, the needle bottle and a block of slides with the identification tag.

blood can be maneuvered into a circular spot about half an inch in diameter at one end of the slide. This must dry thoroughly without causing irregularity in the film margin else time is lost in examining the protrusions that form. The film must be at least a quarter of an inch away from the end of the slide to permit the mechanical stage to cover the entire film without interference from the objective. The only thing that fastens this film to the slide is thorough drying. The slide is stained standing on end to save staining solution and prevent the collection of debris on the film.

Field equipment must be assembled to meet the needs for the day. One must have some knowledge of how many people or animals are to be included in a survey in order to provide sufficient slides. In the case of people, squares of gauze and a bottle of 70 per cent alcohol are needed to clean the skin of the ear, finger, or toe from which blood is to be taken. A two-ounce bottle of alcohol with a No. 5 Hagedorn needle inserted in the cork makes a good blood-lancet. Each time the cork is replaced the needle is submerged in

alcohol thus preparing it for the next person. One should dip the blood-lancet needle up to the cork in paraffin to prevent rusting if it is not in daily use. A wax pencil is required to number the slide on the end not occupied by the film. A lead pencil is wanted to record these numbers in a field book with the name, age, etc., to follow.

Slide boxes with a capacity of 25 slides are used and on these boxes are entered the village, school or organization with the first and last numbered slides. These boxes should be held tightly closed by an elastic band and kept standing in a vertical manner until the blood films are quite dry. To do this, while a slide box is being filled, I use a right-angled stand made of blocks two inches thick. The upright block is the same height and width of a slide box. Another block 4 inches long and the same thickness and width is nailed to the bottom of the upright piece. This makes the base that rests on the table or stand. The opened slide box is then strapped to the upright by an elastic band. This keeps the box level so the films can dry without running. On removing the slide box from this stand it is placed in the same vertical position in the carrying case.

*Field Case.*—The use of leather cases has been abandoned, since the field cases are subjected to all manner of transportation and to heavy rains. This laboratory uses a metal case 8 by 9 by 16 inches with a leather handle similar to that of a suitcase. This case will hold all equipment needed for 400 people or animals. These cases are really carpenter's tool kits made by the Kennedy Manufacturing Co. at Van Wert, Ohio, and Chicago, Ill. They come with a tray but we discard it. These cases are very much less expensive than any made of other materials and they stand severe usage as well as being rain-proof.

*Animal Surveys.*—The only variation in field equipment for these surveys is the addition of a scissors to clip hair off the point of an ear, or to expose the tip of a tail in small animals. A scalpel is also added. This is drawn lightly across the point of the denuded ear and then by pressure drops of fairly clean blood can be removed by contacting the slide. Always wipe the scalpel with alcohol before use on the next animal and apply iodine to the ear wounds. In some animals like the sloth and bat, a toe nail or thumb nail must be cut off to secure blood.

*Source of Blood for Film.*—There is some divergence of opinion as to the most suitable place from which to draw the blood. The writer, for field work, prefers the lobe of the ear as being less sensitive, cleaner, out of sight of the patient and as having some degree of capillary stasis. Parasitized cells are believed to be more abundant in such a system of capillaries. When very young babies are to be included in a survey, it is better to use the ball of the great toe. Many use, as a routine measure, the skin near the base of a finger nail. The Daland's blood-lancet is a very dissembling little instrument that does not necessarily require sterilization. It can easily be covered up with the same gauze used for cleansing the area to be punctured and the puncture is made with a firm quick stab without the patient knowing what moment it is to occur. This is an important point in the management of

nervous people. I have used this lancet on over 6,000 patients without the occurrence of local infection. Care should always be taken to dry the skin and to clear the puncture of any alcohol since blood expressed through or over an alcohol wet surface will fix the cells, and they will not lake as thoroughly as they should. A dry skin allows discrete drops of blood to form at the site of the puncture, and these are sure to be cleaner than blood rubbed off the skin. The blood specimen is stirred or maneuvered into a circular spot about the size of a dime. It is then given its place in the slide box and dried thoroughly before the vertical position of the box is changed. The field notebook will receive the same number given the slide and identification data will be entered on the same line with the number. If care is taken to secure a large drop of blood, there will be no need to stir it into a spot the size of a dime, since it will spread to the proper size if given a level position. Some place the slides with the blood film upside down in the box and place the wax pencil number on the other side at the opposite end. I like the number and the film on the same side of the slide but, of course, at opposite ends. The blood film end of the slide should always occupy the same side of the slide box else it will be difficult to block the slides in 25 lots for the staining bath. Much time is saved and better results are obtained if sufficient blood has been spread in a uniform manner and in a discrete spot about half an inch in diameter. Search the entire film, do not depend on the *thickest focus in the film to always supply the information you seek.*

When a slide box is filled, it is covered and the lid bound on the box with a good rubber band. The necessary information is written on the side or bottom of the box with a lead pencil. For example: Santa Rosa School, slides 1 to 25 or Santa Rosa School, slides 26 to 50, etc. These boxes are kept in a vertical position in the field case until the close of the day or until the following day. It is always better to stain them within twenty-four hours, since they lake with increasing difficulty after that period of time. We have, of course, used such films even after a month or two of drying but they are not as satisfactory and the blood film is apt to crack in many lines, and segments of the film may even be lost. I have been able to use films that were six months old but they are not desirable films. Animal surveys are frequently done in barns and whether the floor is of dirt or boards it pays to wet down the floor to avoid dust settling in the blood films. It is even more necessary to avoid destruction of the films by flies, ants, etc. Flies can remove a large part of a film in a very short time. Care must be taken to guard against breakage or leakage of the alcohol and iodine bottles carried in the field case, since a whole day's collection may be ruined for the staining and examination. Stock bottles are protected by several vertical and transverse rubber bands which not only serve to keep the corks in a tight position but also protect against the jarring of the bottles against each other during transportation.

Thick blood films will dry in a half hour during the *dry season* but in the *rainy season* it is always better to dry them a half hour by placing the closed boxes in the incubator at 37° C. The lid of a box is now removed and pieces of cardboard one inch square by 1/16 of an inch thick are dropped in the spaces between the slides on the ends where the identification numbers have



been written. It is well to drop one between the box and the first slide as well as between the last slide and the box. These cardboard separators are to provide a staining space between the slides. The lid is now replaced and the box turned wrong side up on the table, the bottom of the box is raised slowly taking note that no slides are fast in their brackets. The slides are then brought compactly together by a thumb and finger and moved to the side of the lid where the separators are then pushed flush with the ends of the slides. This is best done by lightly holding the block of slides while a scalpel blade pushes all separators into good position. These separators add protection to the numbers unless too firm a grip is used while moving them into position. In such a case they smear the wax pencil numbers and make reading difficult. The block of 25 slides is then lifted up to the edge of the lid while a good elastic band is placed about the numbered ends of the slides. A second band should then be applied over the first to guard against the breaking of the one band and the unfortunate release of the block of slides. Some prefer a rubber web for this instead of pure rubber bands. Rubber bands  $3\frac{1}{2}$  inches long by  $\frac{1}{4}$  inch wide are used in our equipment. They are long enough to allow two turns of the band and the firm pressure is desired. This makes a solid block that can be handled with ease and safety. Beneath the rubber bands, a small strip of white card is adjusted and on this card is transcribed the entry made on the slide box such as: Santa Rosa School, 1 to 25. Once the boxes have all been blocked in this manner the blocked slides are ready to be stained.

*Staining Method.*—Giemsa's stain is probably universally used. It is an expensive stain when purchased ready for use and after long periods of storage some of the bottles of stain deteriorate. Its preparation from the powder is so simple, the results obtained so satisfactory and its price so greatly reduced that I see no reason why the stain should not be prepared in any laboratory. We estimate it at 75 cents an ounce (stock stain) when made by us. This laboratory does not use Azur II as is the case with the old formula. I place 2.4 gm. of Azur II eosin (Grübler) in a clean Erlenmeyer flask and then add 200 c.c. of C. P. anhydrous glycerin. The flask is covered with a well-fitted cork and then placed in a water-bath at 60° C. for thirty minutes. The flask is shaken once or twice during this period. It is then removed from the bath and 200 c.c. of C. P. methyl alcohol are added and after mixing the contents the flask is again placed in the hot water-bath for another half hour, shaking it once or twice during this period. It is then removed and left in an incubator overnight at 37° C. Next day it is passed through filter paper and stored in 200 c.c. bottles that have been thoroughly cleansed, washed with methyl alcohol, and dried before use. These are well stoppered and kept in the dark. I use equal parts of glycerin and alcohol rather than the larger amount of alcohol advised by some. The liberal use of alcohol is undesirable since thorough laking of the cells is the main object in thick film work. Everything used in the preparation of a stock of Giemsa stain should be as clean as the stock bottles in which it is stored. I use a set of glassware exclusively for this work. Application of the stain requires a dilution of this Giemsa stock

in a proportion of 1 c.c. to one ounce of water. Pour this mixture back and forth about four times and then consider the solution ready for use. The blocked slides are placed in the staining dishes resting on the film ends of the slides with a quantity of stain just sufficient to cover the films. Our service uses the rectangular staining dish 34 by 59 by 82 mm. inside measurement and these hold  $3\frac{1}{2}$  ounces of the staining solution and will receive 50 slides. White enameled trays  $1\frac{1}{8}$  by  $2\frac{7}{8}$  by  $7\frac{1}{2}$  inches (inside bottom dimensions) will accommodate 150 slides and use 9 ounces of staining solution. The slides are left in the stain for one and one-half hours, and then very gently dipped in two changes of water like that used for making the staining solution. They are then dried by standing the blocks of slides on paper that absorb water rapidly such as paper towels. We usually leave them overnight but drying can be hastened if necessary. The films must be thoroughly dry before they are examined. The authors<sup>1, 2, 3</sup> of the modified thick-film method that this

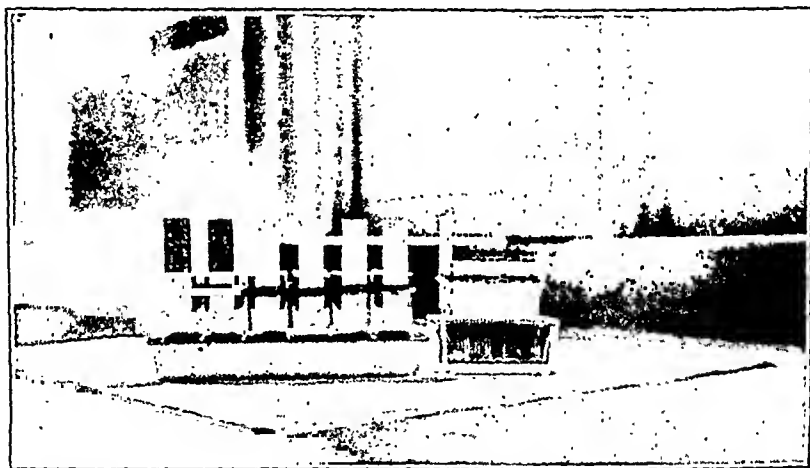


Fig. 2.—An instrument tray with 150 slides and the smaller staining dish with 50 slides in the aqueous solution of Giemsa's stain.

laboratory uses state clearly that the water used for diluting Giemsa stain should be neutral, or only slightly alkaline (pH 7.0 to pH 7.2), and must be nearly or quite free from salts. It has been our experience that clean, fresh rain water and our local city water supply all serve well without treatment for this purpose. Freshly distilled water needs to be corrected. There is always the risk that some accident or repair to the water supply system might cause the loss of a day's collection of slides but thus far it has not and I like our results as well as with water corrected to the proper values. There is considerable latitude in the character of the water to be used but one must stay within reasonable bounds. Much discussion has arisen from the formation of greenish yellow crystals that occasionally cover the film and make examination difficult. This accident will not happen if the staining dishes are kept thoroughly clean between their periods of use.

The complete examination by a trained technician of a well-stained thick film a half inch in diameter will require about three and one-fourth minutes.

There are three factors, at least, that must be taken into consideration in an efficient examination of a thick film, these are as follows: (1) one must be acquainted with the parasites for which he is searching, (2) the entire film should be searched, (3) a proper light and magnification must be employed and a sufficient amount of good immersion oil (cedar oil) to cover the search must be applied to the film.

One familiar with thin films stained with an alcohol polychrome stain will find the thick film picture confusing for a time since all the red blood cells are gone. The field contains leucocytes, platelets, shadows of red cells, and parasites. It will not take much time for one who knows the parasites and is acquainted with thin film work to learn the thick film method, and for diagnostic work it is a most desirable method. There is more upon which to base an opinion than the sparse findings of a thin film. Both methods have their particular points of value but for mass survey work the thick-film method of today fills a most important need. More mixed infestations of malaria, scanty trypanosomal and spirochetal carriers are being found and in wild animal life it is uncovering many facts that are desired in the way of the incidence of filariasis,<sup>4</sup> trypanosomiasis,<sup>5</sup> spirochetosis,<sup>6</sup> piroplasmiasis, anaplasmosis, etc. Naturally the different species of parasites vary a great deal in the number that may be found in a film. The monkey spirochete at the apex of an acute attack may show a countless number to a microscopic field while a trypanosome like *T. cruzi* may never reach a figure above 40 to a field and certain microfilaria may show but a few to the entire film. Scant infections are far more frequently found with this film technic. The writer and his fellow technicians have, during the past four years, examined from man and animals about 200,000 thick films. This diagnostic method is capable of just as wide use in veterinary medicine as it is in human medicine and affords an excellent yardstick to measure the results of sanitation and malaria control. The chief purpose of this thick film method is the diagnosis of parasites in mammalian blood. It is not intended for a study of the characteristics of these parasites or their relationship to the red blood cells.

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## THE BIOLOGIC DIAGNOSIS OF TERATOMA TESTIS\*

SEWARD E. OWEN, PH.D., HINES, ILL.

IT HAS been shown by several workers, Zondek,<sup>1</sup> Heidrich and Fels,<sup>2</sup> Kantrowitz,<sup>3</sup> Lilienthal,<sup>4</sup> Paschkis,<sup>5</sup> Hady,<sup>6</sup> Bollag,<sup>7</sup> Weinstein and Schofield,<sup>8</sup> Johnson and Hall<sup>9</sup> and Ferguson<sup>10</sup> that the sex hormones prolan A and prolan B appear in the urine of men having teratoma testis. Of the above cited references all but Zondek and Ferguson are single case reports. Ferguson<sup>10</sup> classifies the malignant tumors of the testis that may cause the appearance in the urine of man of the prolan hormones as follows: (A) chorionepithelioma, (B) embryonal adenocarcinoma, (C) embryonal carcinoma with lymphoid stroma, (D) seminoma and (E) teratoma with adult features; the amount of hormone in these groups decreasing in the order given.

The work of Ferguson<sup>10</sup> indicates that a suitable test for the quantitative existence of these hormones in the urine is possible. At the time of his report we were employing rabbits in a similar test with somewhat similar results. The use of immature virgin female mice as employed by Ferguson simplifies the problem of handling, feeding and injecting animals. Employing urine from the same specimen and appropriately injecting a series of rabbits and a series of mice, we found from 22 to 25 mouse units as the equivalent to a minimal effective rabbit dose. Due to the advantages of using the smaller animal we now employ mice for the routine tests and reserve the rabbits for quick approximation when time is an important factor. In all cases the quantitative mouse test should be made.

### PREPARATIONS AND TESTS

The diagnosis of pregnancy by means of the Aschheim-Zondek reaction is discussed in many textbooks and the methods of conducting the test are well known. The test as conducted for pregnancy, however, while essentially similar to that for teratoma, does not offer a quantitative value as to the amount of hormone or hormones in the urine. A quantitative test in teratoma is essential in order to obtain consecutive readings which will indicate a decrease or increase in the amount of hormone present when subsequent urine samples are tested.

Fresh morning urine and extracts of urine prepared after the methods of Zondek are employed. Injections are made intravenously in rabbits and intraperitoneally in mice. The extracts employed were made as follows: 50 c.c. of urine acidified to litmus by the use of acetic acid are placed in a precipitating jar and 250 c.c. of 95 per cent ethyl alcohol are added. The mixture is set in the ice box overnight and the resultant precipitate is obtained by centrifugation. The precipitate is then washed with about 50 c.c. of oxide-free ether.

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Received for publication, March 2, 1934.

The precipitate is again obtained by centrifugation and decantation and is allowed to dry. The dry precipitate is then taken up in an aliquot part of water. In our work we commonly use 5 or 10 c.c. of distilled water; thus we have an extract that represents either a concentration of 10 or of 5. Not all of the precipitate is soluble in the distilled water, and it is customary to shake the watery suspension well and allow it to stand at least an hour but preferably overnight in the ice box, after which the residual precipitate may be removed from the watery solution by centrifugation. The supernatant fluid is then labelled and is ready for use.

Immature, virgin, female mice are employed as follows: six animals are selected and tagged or otherwise marked 1 to 6. Mouse number one receives 0.1 c.c. of fresh urine in each of 5 doses spaced over a period of forty-eight hours. Mouse 2 receives 0.2 c.c. of fresh urine in each of 5 doses which are similarly spaced. Mouse 3 receives 0.4 c.c. of fresh urine in each of 5 similarly spaced doses. Mouse 4 gets 5 doses of the 5x extract, 0.1 c.c. per dose. Mouse 5 receives five 0.2 c.c. doses of the 5x extract. Mouse 6 gets five 0.4 c.c. doses of the 5x extract. The 5 doses of extract are spaced over a forty-eight-hour period also. This gives a range in total dosage from  $\frac{1}{2}$  c.c. of fresh urine to 2 c.c. of the extract, and if the 5x extract is used, the highest dosage represents 10 c.c. of urine.

Readings are made on the ovaries about ninety hours from the first injection time. The ovaries are observed grossly and then removed for histologic section preparation. The sections provide us with a reference slide when subsequent tests are made on the same patient. The observations from the gross observations and from the sections are tabulated and translated into mouse units for purposes of record. The following description will explain how this is accomplished; the ovarian changes occurring in the test animals when injected with urine from teratoma patients are: first, a swelling and hyperemia of the graafian follicle accompanied by the formation of a distinct cumulus oophorus. Second, the formation of a massive hemorrhage within the ripened follicle, the so-called corpora hemorrhagica. Third, the formation of corpora lutea atretia. This latter reaction is quite easily distinguished under higher magnifications. Prolan A is said to be responsible for the first two reactions while prolan B is said to cause luteinization. Zondek<sup>1</sup> has shown that prolan A and prolan B exist in the urine of pregnant women in the ratio of 5 to 1. Ferguson<sup>10</sup> has reported that this ratio also holds for urine from males having teratoma. Our work also has shown that whenever Reaction 3 (corpus luteum) occurs, it is possible to get Reactions 1 and 2 with one-fifth to one-sixth as much urine.

The quantitative interpretation of results is as follows: positive cumulus oophorus formation with corpus hemorrhagica in Mice 1 to 6 indicate respectively 2,000, 1,000, 500, 400, 200, and 100 mouse units of hormone per liter of urine tested. It is not essential that corpus hemorrhagica be seen, as this reaction is not of lasting caliber. Reactions which represent cumulus oophorus formation, corpus hemorrhagica formation and corpus lutea atretia when occurring in Mice 1 to 6 indicate in respective order the following number of mouse units, 10,000, 5,000, 2,500, 2,000, 1,000, and 500.

The same reactions are observed in rabbits when 22 to 25 times as much urine or its extract equivalent is injected intravenously. Readings on the rabbit ovaries can be made twenty-four hours following the injection and but one single injection is employed in the rabbit test. The test is done in a manner similar to the Friedman modification of the Aschheim-Zondek test for pregnancy. If but one rabbit is employed for each patient, then the result will only indicate the presence or absence of from 22 to 25 mouse units of hormone in the dosage given, since the intravenous dosage would rarely be over 20 c.c. of urine. The test has its limitations even as an index as will be seen from Table I.

TABLE I  
TEST RESULTS

RABBIT-MOUSE CORRELATION VALUES INDICATING LIMITATION OF RABBIT TEST AS TO DOSAGE EMPLOYED

CASE	RABBIT TEST	MOUSE TEST (MOUSE UNITS)
A	Negative	500
B	Negative	200
C	Questionable	2,000
D	Negative	1,000
E	Negative	2,000
F	Negative	500
G	Positive	2,500
H	Not run	1,000
I	Negative	100
J	Not run	1,500
K	Not run	2,000
L	Not run	2,000
M	Not run	200
N	Not run	Neg.
O	Positive	16,000

Rabbit test in this series all by single intravenous injections of 10 c.c. of fresh urine. Mouse units are calculated to liter of urine.

To compensate for the units of hormone per liter, the twenty-four-hour specimen volume is obtained and if any great variance in volume exists in subsequent tests on the same patient the hormone unitage total is adequately corrected. It is believed from the results that the amount of hormone remains fairly constant, tending to increase, of course, in untreated patients, regardless of the urine volume per day.

Occasionally patients with teratoma subjected to the test will show more than 10,000 mouse units per liter, viz., in Case 10. One single injection of 0.3 c.c. of urine produced all three reactions in a mouse. The interpretation here was at least 16,000 mouse units per liter of urine. A grave prognosis was given in this case. Before effective treatment could be employed or another test obtained, the patient had succumbed to the disease. This patient reported after considerable metastases had occurred and was critically ill at the time of admission. Autopsy confirmed the extensive metastatic involvement suggested by the test.

#### RESULTS OF THE TEST

A few case summaries are included to show the applications of the test. At present over 40 cases of teratoma are being followed by means of the test.

Since it has been shown by Zondek and by others that prolan A and prolan B do not occur in normal male urine, the observance of any of the three reactions mentioned are significant when obtained with male urine injections into either rabbits or mice.

Irradiation of the teratoma mass or of metastases from it reduces definitely the amounts of prolan A and prolan B in the urine, therefore the test has a use in diagnosis. Not only will the increases or decreases of hormone be evident in properly conducted tests but the effectiveness of treatment may be followed accurately. Postoperative tests will show if secondary areas are present. If such areas exist, then the test will indicate whether postoperative irradiation has effectively removed danger. From the cases listed and others, it is believed that if the test is positive preoperatively, rather strenuous irradiation should be applied before operative removal of such tumors. Most of the cases presented to us have had previous treatment, either surgery or irradiation but generally surgery. In these and in other cases the recognition of the presence of metastatic nodules, when such growths are not palpable, is extremely important.

#### FOLLOW-UP SYSTEM

A study of our records reveals the facts that a great many of our teratoma patients live great distances from the facility. It has been difficult to get such patients to report regularly for checking of physical condition and examination, and of course, under such circumstances it is difficult to control treatment. Our present system of follow-up on such patients now includes a mailing tube system for preserved urine specimens. We are attempting to obtain tests on all teratoma patients at least every two months. Patients at a distance from the hospital merely send by mail a sample of urine in a special case which is provided for the purpose. These preserved urine samples are then tested and the test results reviewed by members of the Tumor Clinic who recommend appropriate treatment or subsequent follow-up. Good cooperation on the part of the patients has been the result of this system. Considerable economy is also effected by employing the system, for often transportation expenses can be saved, and naturally hospitalization is not essential for patients with consistently negative results by the test. Since it is commonly accepted that the correct diagnosis of teratoma is made in less than 15 per cent of such cases presented for observation, it is felt that the test applied as a routine procedure in such suspected cases would result in improvement in the prognosis and number of cures by either irradiation or surgery or both.

Following irradiation or surgery, the test is often questionable until ten days or two weeks after the procedure; supposedly the hormones are not at once flushed from the system or the pituitary ceases the overproduction of them immediately. If the irradiation or other treatment is effective, however, the test when made two weeks after such treatment shows this by a reduced amount of hormone in the urine. Case 8 presents such a condition where the test was run too soon after the orchidectomy; a subsequent test in this case was recommended so as to check the result.

## SUMMARY

1. Confirmation of Ferguson's results on the biologic testing for teratoma is offered.
2. A method for follow-up on such teratoma patients is presented.
3. Ten case summaries from a group of 40 teratoma cases are offered as typical examples of the test applications.
4. The importance of preoperative irradiation is stressed.
5. The necessity of consecutive checking, by the test, of such patients having teratoma is outlined.

## CASE SUMMARIES

CASE 1.—White, aged thirty-seven. Injured left testicle in 1925 and again in 1929. Left orchidectomy in 1933 at another hospital. Histologic diagnosis: adenocarcinoma. No palpable glands nor abdominal masses on admission here. Deep x-ray to stump, abdomen, and mediastinum followed by a prolan test which showed 500 M.U. Repeat prolan test two months later gave 200 M.U.

CASE 2.—White, aged thirty-four. No history of injury. Gradual swelling of left testicle up to February, 1933, when a left orchidectomy was done at another hospital. Histologic diagnosis: cancer. Entered here March, 1933, with drainage from scar, inguinal glands palpable. Prolan test showed 1,000 M.U. Deep x-ray therapy to the abdomen and inguinal gland region. Subsequent prolan test was 500 M.U. Further treatment by deep x-ray to the mediastinum was given on suspicion. Supraclavicular area treatment refused.

CASE 3.—White, aged forty-four. No history of injury. About three years ago noted swelling of left testicle. In June, 1932, left orchidectomy at another hospital. Weight loss at the time was about 30 pounds. Transferred here for further treatment. Mass in upper left quadrant of abdomen, no other adenopathy. Chest was negative. Deep x-ray to the left scrotal stump and abdomen was given. Subsequent prolan test 3,000 M. U. Treatment by x-ray at a later date followed by prolan test of 2,000 M.U.

CASE 4.—White, aged forty-five. About ten years ago, had injury to right testicle; swelling resulted which subsided until a year ago when swelling of the right testicle again became apparent. Back pains, pulling sensations in right groin. Right orchidectomy at another hospital in February, 1933. No irradiation was given at the time. On admission here was given deep x-ray therapy to right scrotal stump and to abdomen. The prolan test following treatment was 2,000 M.U. on January, 1934.

CASE 5.—White, aged thirty-nine. History of testicular injury in June, 1933. In March, 1933, biopsy at another hospital showed suspected malignancy. Right orchidectomy was done at the same outside hospital. On admission here in April, 1933, he was given deep x-ray to the stump and abdomen and released. On readmission in August, 1933, no adenopathy was apparent. On another admission in October, 1933, the prolan test was negative. The follow-up specimen of January, 1933, shows 200 M.U. by prolan test.

CASE 6.—White, aged fifty-three. Injured left testicle in service; swelling followed by receding at that time. May, 1932, swelling of left testicle prompted hospitalization in August, 1932. In this outside hospital the serotum was tapped and a left orchidectomy was done. Histologic diagnosis was carcinoma of testes. No palpable glands. Admitted here September, 1932. No palpable glands were noted. Irradiation to scrotal stump and abdomen. Follow-up specimen of January, 1934, shows 1,000 M.U.

CASE 7.—White, aged forty. Injury to right testicle on January, 1932, swelling continued. Admitted to another hospital where right orchidectomy was done November, 1932, with no other treatment. Admitted here January, 1933. Adenopathy negative, chest ques-



tionable. Irradiation deep x-ray to scrotal stump, abdomen, and mediastinum. Readmitted August, 1933. Irradiation to mediastinum over suspected chest nodes. Follow-up specimen of January, 1934, shows 1,500 M.U. by prolan test.

CASE 8.—White, aged thirty-four. No history of injury. December, 1932, gradual swelling of left testicle. December, 1933, left orchidectomy at another hospital with no other treatment. Admitted here December, 1933, where no adenopathy was found and irradiation was not advised. The prolan test of Dec. 24, 1933, showed less than 100 M.U.

CASE 9.—White, aged thirty-four. Formerly admitted here for pulmonary tuberculosis and tuberculosis of the gastrointestinal tract, as well as for heart trouble. No previous history of injury. In 1931 first noted swelling of right testicle. On admission here in May, 1933, a right orchidectomy was done. The histologic diagnosis was teratoma. Irradiation treatment given to postoperative scar, abdomen, mediastinum, right and left scapular regions. Prolan test of October, 1933, was negative.

CASE 10.—White, aged forty-five. No history of injury. Had a right orchidectomy at another hospital in January, 1933. Two months later he noted abdominal pains. Admitted here October, 1933, with diagnosis of abdominal tumor, carcinoma of lungs, nodes in left supraclavicular region. Prolan test showed 16,000 M.U. Irradiation therapy was attempted but the critical condition of the patient precluded the continuance of such treatment. Patient died a few days after admission. Autopsy showed extensive metastases to the abdomen, lungs, neck lymph glands, and to other lymph glands. The metastatic nodules were of the teratoma type.

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## A NOTE ON FRIEND'S METHOD FOR THE ESTIMATION OF CHLORIDES\*

JOHN E. HEARN, NEW YORK, N. Y.

WHEN Herman Friend's method† for the direct titration of chlorides in blood appeared, it seemed for simplicity to leave nothing to be desired. However, the first estimation made with the new method (on a specimen of normal blood) was considerably higher than had been expected.

The silver nitrate solution was then titrated against a 0.5 per cent sodium chloride solution and was found to be of lower concentration than theory demanded. The source of such errors is of little import as compared with the consideration that they are prone to occur, and to affect the results of a series of determinations if not detected.

The factor 1.17 in Friend's equation had to be reduced, and his table was consequently rendered useless. It was then deemed best to make a solution of silver nitrate (2.906 gm. per liter) of which 1 c.c. would represent 1 mg. of sodium chloride. With this change, the table is not needed.

The modified procedure is as follows:

To 1 c.c. of plasma 3 c.c. of the aluminum hydroxide cream is added after diluting the plasma with 10 c.c. of distilled water. The volume is then made up to 25 c.c. with water. A drop of caprylic alcohol may be used to dispel the foam, thus increasing the accuracy of measurement. After mixing and letting stand for ten minutes, the mixture is filtered. To 20 c.c. of the filtrate 5 drops of 5 per cent potassium chromate is added, and the filtrate is titrated with the standard silver nitrate of which 1 c.c. represents 1 mg. of sodium chloride. The end-point is the change from yellow to brownish, and it should be the same tint as that reached in the standardization of the silver salt against the sodium salt. When the number of cubic centimeters of the silver nitrate solution used is multiplied by 125, the result expresses the chloride content in mg. NaCl per 100 c.c. of plasma.

The method is applicable to urine also. For this use it is even simpler, as preliminary treatment is usually unnecessary. One cubic centimeter of urine in approximately 25 c.c. of distilled water is titrated without filtering. The number of cubic centimeters required corresponds to the chloride content in gm. NaCl per liter.

It is desired specifically to avoid the implication that Friend's factor 1.17 is either theoretically or practically erroneous. The substitution of the standard solution (containing silver nitrate representing 1 mg. NaCl per c.c.) for the 0.02 normal solution is for convenience in calculation merely. The pub-

\*From the Pathological Laboratory of the French Hospital.  
Received for publication, March 13, 1934.

†Friend, H.: Estimation of Chlorides in Blood, *J. Biol. Chem.* 51: 115, 1922.

lication of the results of titrations of pure chloride solutions in this connection would be a work of supererogation; it would prove nothing except the accuracy or inaccuracy of the chemist doing the work. The comparison made by Friend of results with his own method and with the standard one of McLean and Van Slyke would be duplicated if the modified method were used by a chemist of equal competence, and under the same conditions, for this method is still essentially Friend's.

126 WEST NINETY-FOURTH STREET

## THE DETERMINATION OF URINE CHLORIDES WITH MERCURIC NITRATE\*

C. E. HOLDRIDGE, M.S., AND J. W. CAVETT, PH.D., MINNEAPOLIS, MINN.

THE chloride method of Votoček<sup>1</sup> using mercuric nitrate to titrate chloride solutions with sodium nitroprusside as an indicator was applied by Cavett and Holdridge<sup>2</sup> to the Folin-Wu filtrate in the determination of blood plasma chlorides. This method has proved very satisfactory and has been applied to the determination of urine chlorides.

### METHOD

Five cubic centimeters of urine are placed in a 100 c.c. volumetric flask and diluted to volume. A 5 or 10 c.c. aliquot is taken for the titration using the same reagents and the exact procedure as given in the plasma chloride method for the titration of the Folin-Wu filtrate.†

Urines which contain protein give a turbidity with the mercuric ion which interferes with the end-point of the titration. Thus, with such urines, 5 or 10 c.c., depending on the chloride content, are placed in a 100 c.c. volumetric flask and 5 c.c. of 10 per cent *sulphosalicylic* acid are added. The flask is placed in a boiling water-bath for a few minutes, cooled, and diluted to volume. The contents of the flask are filtered through a dry filter. If the filtrate shows any turbidity it is returned to the filter until it comes through clear. The titration is then carried out on a 5 or 10 c.c. aliquot.

Comparison with the Volhard-Arnold silver nitrate method as given by Hawk and Bergeim<sup>3</sup> shows the error to lie within one per cent.

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\*From the Laboratory of Physiological Chemistry of the University of Minnesota.  
Received for publication, March 5, 1934.

†One cubic centimeter of the mercuric nitrate solution is equivalent to 1 mg. NaCl. The titration corrections given in Table I of the plasma chloride method are used.<sup>2</sup>

# A NEW PROCEDURE FOR INTRODUCING SOLUTIONS DIRECTLY INTO THE SMALL INTESTINE OF EXPERIMENTAL ANIMALS\*

STEPHEN MADDOCK, M.D., BOSTON, MASS.

RECENTLY a technic was reported (Maddock, 1932) which permits ligation of the pylorus of animals from the exterior without the complications of general anesthesia. Utilization of this procedure enabled Maddock, Trimble, and Carey (1933) to demonstrate that the absorption of d-glucose from the stomach is either zero or negligibly small.

The present communication describes a procedure which permits the rôle of the small intestine in absorption to be studied separately from that of the other portions of the alimentary tract of the dog. The technic of this is as follows: Under ether anesthesia with aseptic precautions a midline incision is made from the tip of the xyphoid to a point a few centimeters above the umbilicus. The glass pyloric snare is then inserted as described previously (Maddock, 1932). The wound is closed with interrupted silk sutures.

A right rectus muscle-splitting incision is then made and the loop of the duodenum, distal to the lower pancreatic duct (Santorini) is brought to the outside. The edges of the abdominal wall are approximated under the duodenum by placing 1 or 2 sutures through both peritoneum and fascia, in a manner similar to that described by Mann (1921). This allows about 5 cm. of duodenum to lie on the anterior rectus sheath where it is readily accessible (Fig. 1). The skin is then closed over the gut with interrupted silk sutures. Dry dressings are placed over the wound and held in place by a scultetus binder.

The animals recover rapidly from this operation and are usually willing to consume a meal of bread and milk or ground meat by the following day. After from three to seven days they may be used for an absorption test as described below. Somewhat less than 10 per cent of the experimental animals fail to recover their appetites promptly. A few of these show elevation of blood nonprotein nitrogen. All dogs which refused food for several days or whose appetite was poor gave subnormal results in absorption tests, so that a normal appetite appears to be an important criterion of the success of the preliminary surgery.

On the day of the absorption test the skin sutures are removed. The snare is drawn sufficiently taut to close the pylorus. A hollow needle is then inserted into the lumen of the duodenum, and the fluid whose absorption is to be studied is infused through the needle. An alternative procedure which is also feasible, is to make a 5 mm. incision in the duodenum through which a soft rubber catheter is inserted into the lumen. A purse-string suture is then placed about the opening and tied snugly around the catheter to prevent leakage.

\*From the Surgical Research Laboratory of the Boston City Hospital.  
Received for publication, March 8, 1934.

At the end of the experiment the animals are quickly and painlessly sacrificed by injecting sodium amytal (100 mg. per kilo) intravenously. The intestine can then be removed and completely washed out in order to make a quantitative study of absorption from the intestine or of chemical changes which may have occurred during the experimental period.

### RESULTS

In order to test the procedure several types of experiment have been performed. In these the absence of hyperglycemia has been used as a criterion of the normality of the animal during the absorption test.

The animals were given the preliminary surgical preparation just described. On the day of the absorption tests (3 or 5 days after the preliminary

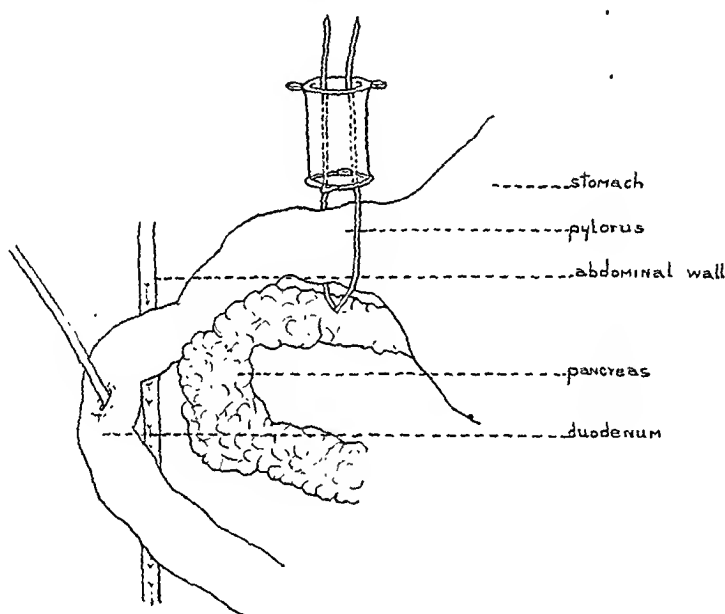


Fig. 1.

operation) the animals (fed eighteen hours previously) were brought into the laboratory and a sample of venous blood (I control) was obtained. The pylorus was then closed and the needle inserted into its duodenum. A second specimen of blood (II control) was procured.

Then with the aid of a Woodyatt pump fluids (water or sodium chloride) warmed to  $37^{\circ}\text{C}$ . were injected directly into the small intestine at constant rates. At fifteen-minute intervals specimens of blood were collected from the saphenous veins. The sugar content of these was determined by the ferrocyanide method of Folin and Malmros (1929).

The data obtained are recorded in Table I. These show that neither the manipulative procedures immediately preceding the injection nor the infusion of considerable volumes of fluids directly into the small intestine provoked a hyperglycemic response of any significant magnitude.

TABLE I

EXPERIMENT NUMBER	I	II	III	IV
Weight of dog, kg.	17.0	15.1	23.5	15.2
Substance ingested	H <sub>2</sub> O	NaCl	NaCl	NaCl
Concentration per cent		0.9	2.5	2.7
Volume, c.c.	800	450	640	820

*Blood Sugar Concentrations, Milligrams Per Cent, During Injection*

I Control	87	85	80	70
II Control	89	92	78	72
15 minutes	92	92	81	74
30 minutes	93	91	82	75
45 minutes	88	92	83	73
60 minutes	90	82	84	69
75 minutes	89	89	82	74
90 minutes	85	87	86	76
105 minutes	90	89	82	74
120 minutes	89	89	85	83

*Corpuscle Volume, Per Cent*

Before injection	47, 48	43, 42	44, 45	52, 52
After injection	46, 46	40, 39	34, 33	52, 53

*Leakage from Intestine into Stomach.*—That the pyloric snare is able to prevent solutions passing backward into the stomach was shown in the following tests. D-glucose (concentrations 3-22 per cent, volumes 100-600 c.c.) was infused directly into the duodenum. After completion of the infusion (which consumed from one to three hours), the content of the stomach was collected and tested for the presence of reducing substances. In a series of 9 experiments the quantities found were respectively 22, 95, 29, 162, 9, 90, 135, 43, and 32 mg. In a previous communication Maddock, Trimble and Carey (1933) have shown that d-glucose is not absorbed directly from the stomach. Therefore the data just presented demonstrates that leakage from the intestine into the stomach need not occur when the procedure presented in the present communication is employed.

The technic described above provides a method for introducing a wide variety of substances into the intestine of an intact animal under conditions which avoid the necessity of general anesthesia or permanent fistulas.

*The Rate of Absorption of Sodium Chloride.*—Incidental to the main purpose of the experiments recorded there was opportunity for a few observations upon the rate at which sodium chloride is absorbed from the intestine. Computations based upon the data of Experiment III show that in this instance the total sodium chloride ingested was 16.0 gm. Of this 1.43 gm. was recovered from the intestine at the conclusion of the experiment. Therefore 14.57 gm. of sodium chloride had been absorbed during two hours. This occurred at the rate of 0.31 gm. per kilo of body weight per hour. In Experiment IV the sodium chloride infused totaled 22.14 gm. The recovery was 9.05 gm. and the absorption rate was 0.42 gm. In Experiment III dosage of sodium chloride was 0.68 gm. per kilo while in Experiment IV it was 1.45 gm. per kilo. In both experiments the concentration of the solution was substantially the same. These observations together with others not included here, present some indication that an increased dosage of sodium chloride produces an

increase in the rate at which this salt is absorbed from the intestine. This is in contrast with the observation of Trimble and Maddock (1934) that the rate of absorption of d-glucose from the intestine of the dog is not significantly altered by variations in its dosage.

This procedure was developed and the accompanying observations were made during the course of an investigation carried on in collaboration with Doctor Harry C. Trimble of the Department of Biological Chemistry, Harvard Medical School.

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### A SIMPLIFIED TECHNIC FOR THE COLORIMETRIC DETERMINATION OF BLOOD CHOLESTEROL

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R. S. FIDLER, B.A., M.D., COLUMBUS, OHIO

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THE inherent technical difficulties in the methods of Bloor,<sup>1</sup> Sackett,<sup>2</sup> and Myers and Wardell<sup>3</sup> for the determination of blood cholesterol have made a simple colorimetric method highly desirable. In 1928 Cornell<sup>4</sup> described a colorimetric technic for the determination of blood and tissue cholesterol based upon the drop precipitation of blood in an alcohol-ether mixture at room temperature. At that time we compared the results obtained by this alcohol-ether extraction with those obtained by the older methods and were able to verify Cornell's statement that extraction by this method is complete.

In following the further directions of Cornell (consisting of washing of the precipitation flask and the precipitate with alcohol-ether, reducing the filtrate volume by distillation and the use of a part of the reduced filtrate for final evaporation) the method was found to be somewhat cumbersome and because of the several steps involved, subject to technical errors.

Separate examinations of the precipitate washings revealed the fact that they contained insufficient cholesterol to give a readable color development by the Burchard Leibermann reaction. We accordingly eliminated the washings and with them the necessity of reduction of the filtrate volume by distillation. We are now using an aliquot part of the original filtrate for the determination, a procedure entirely in accord with other blood chemical determinations in which the Folin-Wu filtrate is used.

<sup>1</sup>From the Department of Pathology, White Cross Hospital.  
Received for publication, March 12, 1934.

In the use of the Burehard-Leibermann reaction for the maturation of color in the chloroform solutions of cholesterol, control of three variable factors is necessary if accurate colorimetric comparisons are to be obtained. Color develops slowly in cold solutions and rapidly in warm solutions; greater intensities of light also accelerate the color development. If conditions of temperature and light are standardized, time of development remains the only variable factor. If chloroform solutions of pure free cholesterol are used as the standard and exposed to the same physical conditions of temperature and light as the unknown samples, the development of color in both standard and unknown are presumably parallel and the exact time of maximum development of color at which colorimetric comparisons are to be made may vary within certain arbitrary limits without introducing clinical errors. If, however, the more convenient artificial standard is used for comparisons, the time factor must be constantly under control in order that colorimetric comparisons may be made at the exact point of maximum color development in the unknown solution. It is obvious that this time will vary with each determination, being influenced by the room temperatures, temperature of the solutions, and the various light conditions.

It is desirable that color maturation progress slowly. In order to obtain slow color development by the control of the variables of light and temperature Cornell directs that the containers be chilled in an ice bath, then placed at an arbitrary fixed distance from a 200 watt lamp in a dark room for forty-five minutes. In 1932 Mirsky and Bruger<sup>5</sup> and later Mirsky<sup>6</sup> directed that the containers be placed in a dark closet at room temperature for five minutes and then for another ten minutes in the refrigerator. It is obvious that the variabilities of room temperature in either procedure would influence the exact time set for comparison of the developed colors, but as these authors were using the natural standards the time factor was of minor importance.

In order to use the more convenient artificial standard we have modified our procedure for color maturation. We now develop color by the Burehard-Leibermann reaction at room temperature throughout and expose the unknown solution to the same light by which the subsequent color comparisons are to be made, in the cups of the artificially illuminated colorimeter itself. By this method repeated colorimetric comparisons are possible during the entire range of color maturation and fading and the exact point of maximum color development is not missed.

#### PROCEDURE

Into a 125 c.c. Erlenmeyer flask place exactly 24 c.c. of a two to one mixture of alcohol and ether. From a 1 c.c. volumetric pipette add drop by drop and with constant agitation 1 c.c. of whole oxalated or heparinized blood. Cork tightly and agitate for two minutes. Filter through a fat-free paper into a stoppered 50 c.c. wide mouth bottle.

Pipette 5 c.c. of this filtrate (an aliquot part) into a 50 c.c. Erlenmeyer flask and evaporate on the "low" heat of an electric hot plate until about one drop of solution remains. Remove at this point to prevent complete drying and overheating with possible discoloration of the residue. Add at once



5 c.c. of chloroform. Cork tightly and allow to stand until it cools to room temperature. The addition of the chloroform to the residue while hot assures complete solution of the cholesterol. When making a series of examinations, the flasks may stand stoppered at this point until it is convenient to develop the color.

When cool add 0.2 c.c. of concentrated sulphuric acid, stopper and cool to room temperature, then add 1 c.c. of acetic anhydride. Allow to stand for two minutes, transfer to the colorimeter cups and compare with the standard every minute until constant maximum readings are obtained, then for several minutes more until the color fades, through three successive readings.

The standard used is National Aniline Company's Naphthol Green B from which a 0.1 per cent aqueous stock solution is prepared. The stock is stable for several weeks but must be diluted fresh before using. If 1 c.c. of this stock is diluted with 19 c.c. of water the resulting color is equivalent to that produced by 0.0004 gm. of pure cholesterol dissolved in 5 c.c. of chloro-

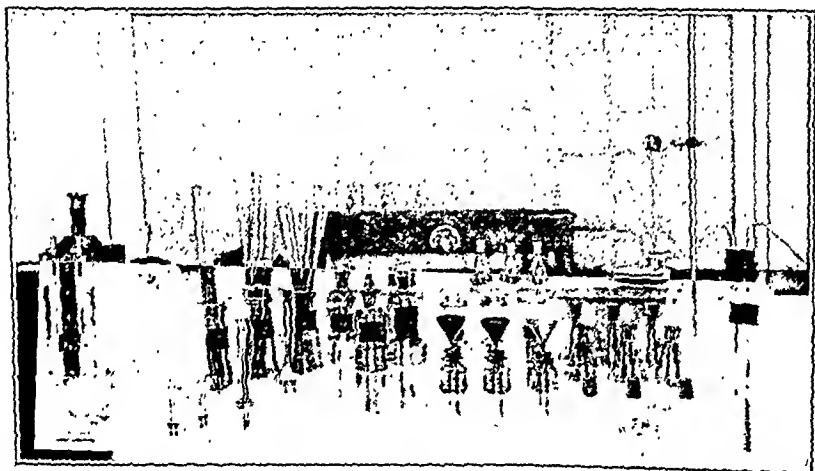


Fig. 1.—Apparatus for blood cholesterol determination, showing precipitation flasks, filtration bottles, evaporation flasks, hot plate, buret for alcohol-ether mixture and the artificially illuminated colorimeter used in these experiments.

form. Since the unknown cholesterol in the 5 c.c. of chloroform solution is further diluted by the addition of 0.2 c.c. of sulphuric acid and 1 c.c. of acetic anhydride it is necessary to add a further 1.2 c.c. of water to every 5 c.c. of the diluted standard. By setting the unknown at 20 on the colorimeter scale and reading the standard the calculations become:  $\text{Standard} \times 10 = \text{Mg. Cholesterol in 100 c.c. of whole blood.}$

The apparatus used by the author is shown in Fig. 1.

#### DISCUSSION

The practical accuracy of the above method is demonstrated by the following check determinations made on four different blood samples:

Sample 1	Determination 1	161 mg.
	Determination 2	160 mg.
	Determination 3	161 mg.
	Determination 4	160 mg.

Sample 2	Determination 1	240 mg.
	Determination 2	239 mg.
	Determination 3	241 mg.
	Determination 4	240 mg.
Sample 3	Determination 1	132 mg.
	Determination 2	133 mg.
	Determination 3	131 mg.
	Determination 4	132 mg.
Sample 4	Determination 1	115 mg.
	Determination 2	114 mg.
	Determination 3	114 mg.
	Determination 4	115 mg.

The variations in the above determinations are within the limits of error inherent in all colorimetric readings.

The control of the variables of time, light, and temperature by constant colorimetric observations of the maturing colors is illustrated by the following readings taken at random from the records of cholesterol determinations. These determinations were made on different days under different conditions of room temperature and represent blood from normal and pathologic cases.

Sample 1	3 minutes	125 mg.	Maximum in 6 minutes
	4 minutes	135 mg.	
	5 minutes	150 mg.	
	6 minutes	155 mg.	
	7 minutes	155 mg.	
	8 minutes	154 mg.	
	9 minutes	152 mg.	
	10 minutes	145 mg.	
Sample 2	3 minutes	88 mg.	Maximum in 12 minutes
	4 minutes	93 mg.	
	5 minutes	95 mg.	
	6 minutes	100 mg.	
	7 minutes	105 mg.	
	8 minutes	110 mg.	
	10 minutes	122 mg.	
	11 minutes	130 mg.	
	12 minutes	131 mg.	
	13 minutes	131 mg.	
Sample 3	14 minutes	125 mg.	Maximum in 10 minutes
	15 minutes	115 mg.	
	16 minutes	105 mg.	
	3 minutes	40 mg.	
	4 minutes	52 mg.	
	5 minutes	80 mg.	
	6 minutes	116 mg.	
	7 minutes	140 mg.	
	8 minutes	164 mg.	
	10 minutes	165 mg.	
	11 minutes	165 mg.	
	12 minutes	163 mg.	
	13 minutes	162 mg.	

The effect of temperature, as well as the practical accuracy of the method, is illustrated by the following colorimetric readings of three separate determinations on a single sample of blood containing an abnormally high amount of cholesterol. In these determinations the acetic anhydride was added to the warm, partially cooled and room temperature chloroform solutions of the cholesterol residue after the addition of the sulphuric acid.

WARM		PARTIALLY COOLED		ROOM TEMPERATURE	
2 minutes	60	2 minutes	40	2 minutes	30
3 minutes	82	3 minutes	74	3 minutes	42
4 minutes	100	4 minutes	100	4 minutes	70
5 minutes	184	5 minutes	170	5 minutes	96
6 minutes	210	6 minutes	192	6 minutes	100
7 minutes	260	7 minutes	222	7 minutes	116
8 minutes	272	8 minutes	240	8 minutes	140
9 minutes	290	9 minutes	253	9 minutes	163
10 minutes	298	10 minutes	273	10 minutes	190
11 minutes	300	11 minutes	287	11 minutes	220
12 minutes	300	12 minutes	298	12 minutes	252
13 minutes	299	13 minutes	300	13 minutes	268
14 minutes	299	14 minutes	300	14 minutes	279
15 minutes	296	15 minutes	301	15 minutes	288
16 minutes	290	16 minutes	300	16 minutes	296
		17 minutes	294	17 minutes	299
Maximum in 11 min.		18 minutes	290	18 minutes	301
				19 minutes	301
		Maximum in 15 min.		20 minutes	302
				21 minutes	301
				22 minutes	300
				23 minutes	297
				Maximum in 20 min.	

## SUMMARY AND CONCLUSIONS

1. A simplified and accurate technic for the determination of blood cholesterol is described. We do not present this technic as entirely new but only as a simplified method which has proved highly satisfactory.

2. By the elimination of unnecessary washings and reduction of excess filtrate volume by distillation, time is saved and accuracy increased. The use of an aliquot portion of the filtrate is quantitatively accurate.

3. The use of naphthol green B as the artificial standard is entirely satisfactory and much less difficult than the preparation of chloroform solutions of pure free cholesterol.

4. By developing color at room temperatures in the artificial light of the colorimeter a series of comparisons can be made throughout color maturation and absolute control of the variable factor of time is attained.

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# PROTEIN ANALYSES IN CEREBROSPINAL FLUID\*

## A COMPARATIVE STUDY OF METHODS

BURNHAM S. WALKER, PH.D., AND HENRY J. BAKST, M.D., BOSTON, MASS.

SINCE the introduction by Denis and Ayer<sup>1</sup> of a rapid and simple method for the estimation of the protein content of cerebrospinal fluid, this particular analysis has acquired considerable diagnostic import. It is generally accepted by all clinical analysts, as far as our inquiries have extended, that the Denis and Ayer method is entirely satisfactory for this measurement, being both rapid and, within certain limits, exact.

In a later communication, in which the original Denis and Ayer technique is slightly modified, Ayer, Dailey and Fremont-Smith<sup>2</sup> state that "The exact magnitude of the error inherent in the method has not been determined, since there is no more accurate method to use as a check on individual samples of normal fluid." They present comparative figures between the modified Denis and Ayer method, and macro- and micro-Kjeldahl determinations in which the protein nitrogen is estimated *by difference*; that is by subtraction of the non-protein nitrogen from the total nitrogen. As they are careful to point out, such an indirect method permits a possibility of rather large accumulation of error. The actual percentage error in these comparative figures ranges from 2.6 per cent to 17.6 per cent, with a mean value of 8.6 per cent, which it should be remembered represents the algebraic sum of the errors of both analyses.

In view of the wide clinical use of the Denis and Ayer method, and the admitted question as to its exactness, it has seemed advisable to attempt to check the results obtained with the Denis and Ayer method compared with two other methods, both based upon entirely different analytical principles. One of these is a micro-Kjeldahl procedure, in which the protein nitrogen is determined by *direct* digestion of the precipitated protein. This method, already published by Walker and Sleeper,<sup>3</sup> has been shown by them to agree with macro-Kjeldahl determinations on dilute egg albumen solutions with a maximum error of 3 per cent. The other is a colorimetric method, dependent on the production of a color by reducing a protein-phosphomolybdic acid precipitate.

### EXPERIMENTAL

*Denis and Ayer Method.*—It was carried out according to the modified procedure of Ayer, Dailey and Fremont-Smith,<sup>2</sup> and using a Bausch and Lomb biologic colorimeter fitted with the special small cups and plungers as recommended in that same communication.

*Micro-Kjeldahl Method.*—It was carried out according to the procedure of Walker and Sleeper,<sup>3</sup> and using an ordinary biologic colorimeter.

\*From the Evans Memorial (Massachusetts Memorial Hospital) and the Fifth Medical Service, Boston City Hospital.

Received for publication, March 19, 1934.

*Colorimetric Method.*—To 1 c.c. of fluid in a small test tube (about 5 c.c. capacity) is added 1 c.c. of 1 per cent phosphomolybdic acid in normal sulphuric acid. After mixing and standing for fifteen minutes, this is centrifuged, the supernatant liquid decanted as completely as possible and discarded. The precipitate is resuspended in water, again centrifuged and the supernatant liquid discarded. (This washing is essential to remove excess of unprecipitated phosphomolybdic acid.) To the washed precipitate is now added 1 c.c. of fifth normal sulphuric acid, 1 c.c. of 2 per cent hydroquinone, and after shaking, 1 c.c. of the carbonate-sulphite solution used in the Bell and Doisy<sup>4</sup> method for phosphates (15 per cent sodium carbonate and 3 per cent sodium sulphite). The color is read in the colorimeter fitted with small cups against a standard similar to that used in the Denis and Ayer method, treated in the same manner as the unknown fluid. The colorimetric reading should be made promptly, as the color has a tendency to fade rather rapidly.

## ANALYTICAL RESULTS

It is first necessary to show that the new colorimetric method yields results comparable to the macro-Kjeldahl, which still remains our standard of protein determinations. Table I shows the results obtained with dilutions of a stock protein solution, made up and standardized according to the procedure of Denis and Ayer in preparing their protein standard.

TABLE I

MG. PROTEIN PER 100 C.C. (MACRO-KJELDAHL)	MG. PROTEIN PER 100 C.C. (COLORIMETRIC)	PERCENTAGE ERROR
159.0	155.0	2.5%
119.0	121.0	1.7%
79.5	79.0	0.6%
59.5	59.0	0.8%
23.8	22.3	6.3%
19.8	18.9	4.5%

Inspection of Table I shows that with moderately increased amounts of protein this method gives results which are accurate within the limits of personal error in colorimetric reading. In the lower ranges the color is more difficult to apprehend, and the error is somewhat, although not prohibitively, greater.

The three methods were next compared on three samples of pooled cerebrospinal fluid, the results of which are shown in Table II.

TABLE II  
POOLED CEREBROSPINAL FLUID  
Mg. Protein per 100 c.c. Fluid

BY MICRO-KJELDAHL METHOD	BY DENIS AND AYER METHOD	BY COLORIMETRIC METHOD
33.5	32.5	33.0
32.4	30.5	32.0
22.1	20.5	21.7
<i>Percentage Recovery (Assuming Micro-Kjeldahl as 100 Per Cent)</i>		
100	97	98.5
100	94	99.0
100	93	98.0

Inspection of Table II shows that on the same fluid the three methods give essentially the same results. Both the Denis and Ayer method and the new colorimetric method yield results slightly, but hardly significantly lower than the micro-Kjeldahl. By its simplicity, the Denis and Ayer method recommends itself as the most satisfactory for clinical use. The micro-Kjeldahl procedure remains, we believe, the standard procedure for exact determinations. The colorimetric method may perhaps find a use as a rapid check method, but we feel that it has already served its most important purpose in proving, we trust conclusively, that the Denis and Ayer method is valid and dependable.

#### SUMMARY

Three methods for the determination of small amounts of protein, all found accurate when tried with dilute protein solutions, have been applied to samples of pooled spinal fluid, and have yielded similar results. The method of Denis and Ayer being the simplest, is recommended for use in clinical spinal fluid protein estimations.

#### REFERENCES

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### FURTHER OBSERVATIONS ON THE COLLOIDAL CARBON FLOCCULATION TEST IN SPINAL FLUID\*

PURCELL G. SCHUBE, M.D., BOSTON, MASS.

IN AN earlier publication<sup>1</sup> the method of performing a colloidal carbon flocculation test in spinal fluid was described in detail. Since that publication the work on the method has been continued and although the fundamental principles have remained as before, several minor alterations have been introduced. It is the purpose of this paper to describe them and the results obtained.

#### ALTERATIONS OF THE TEST

In the course of the work upon the test, it was found that a set of six tubes was unnecessary except in cases of meningitis in which the fluid was not sterile. Four tubes in the set were sufficient for all other spinal fluids. Into each tube was placed 1 c.c. of distilled water. In the first tube of the set was placed 1 c.c. of spinal fluid. It was thoroughly mixed and 1 c.c. of this mixture was

\*From the Psychiatric Clinic, Boston State Hospital.  
Received for publication, April 6, 1934.

transferred to the second tube of the set. This process was repeated through the four tubes, the last 1 c.c. being discarded. Into each of the four tubes 0.1 c.c. of 0.1 per cent oxalic acid solution was measured. Each tube was inverted several times to mix. Then into each tube was added 0.4 c.c. of 1 per cent suspension of colloidal carbon. Each tube was again inverted several times to mix.

Dual controls were set up with each spinal fluid, one with oxalic acid and one without oxalic acid in order to establish comparative extremes.

The set was kept at room temperature and read twelve hours later as has been described previously.

# RESULTS

This test has been performed upon 552 spinal fluids. Of these the distribution was:

General paralysis	50 cases	Epilepsy	26 cases
Tabeo-paresis	22	Senile deterioration	18
Cerebrospinal syphilis	17	Psychoneurosis	18
Meningovascular syphilis	12	Psychopathic personality	17
Schizophrenia	60	Organic brain disease	16
Cerebral arteriosclerosis with psychosis	50	Posttraumatic constitution	14
Manic depressive psychosis	48	Without psychosis	12
Alcoholic psychosis	42	Hydrocephalus	11
Mental deficiency	36	Multiple sclerosis	11
Senile psychosis	33	Cerebral hemorrhage	9
Toxic psychosis	27	Diabetes insipidus	3
		Total	552 cases

In these cases the carbon test was positive only in the spinal fluid of the cases of neurosyphilis. In all others it was negative. No relationship could be established between the test and the spinal fluid protein, proteose, sugar, chlorides, pH, or cell count.

# DISCUSSION

Colloidal carbon when in suspension carries a negative electrical charge. Oxalic acid in solution carries a positive electrical charge. When these two are mixed the negative charge on the carbon particle is neutralized, the particles due to decreased surface tension aggregate and flocculate out, leaving a solution whose clearness depends upon the degree of flocculation.

When oxalic acid is added to normal spinal fluid, the positive electrical charge is absorbed completely by the existing free negatively charged particles in the spinal fluid so that when the colloidal carbon is added its charge remains unchanged and the carbon remains in suspension; the mixture remaining black.

But in neurosyphilitic spinal fluid there do not exist sufficient "free" negative electrical charges to neutralize the positive electrical charges of the oxalic acid, so that when the oxalic acid is added its positive electrical charges to a large extent remain unchanged. When colloidal carbon is added to this mixture, its free negative electrical charges are neutralized by the positive electrical charges of the oxalic acid, and the carbon flocculates out. The protective qualities of the protein in the spinal fluid are made inert by the addition of distilled water to the mixture.

Another way to explain this phenomenon more easily is thus:

In the test an organic sol, an inorganic sol, and an electrolyte are used. When the organic sol is mixed with the inorganic sol of the same charge of particles, the organic sol protects the particles of the inorganic sol from flocculation by electrolytes. The organic sol is the spinal fluid; the inorganic sol is the colloidal carbon; and the electrolyte is the oxalic acid. In neurosyphilitic spinal fluids the activity of this protective organic sol is decreased in dilutions corresponding to Tubes 1, 2, and 3.

#### SUMMARY

1. The spinal fluids of 552 individuals have been examined by means of the colloidal carbon flocculation test.
2. Flocculation occurs only in the spinal fluids of neurosyphilis.
3. The possible mechanism of the test is described.

#### REFERENCE

1. Schube, P. G., and Harms, H. E.: A Colloidal Carbon Flocculation Test in Spinal Fluid. Preliminary Report, *J. LAB. & CLIN. MED.* 18: 65, 1932.

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## A MICROMETHOD FOR THE DETERMINATION OF BLOOD NONPROTEIN NITROGEN\*

H. IRVING, L.T., AND J. C. FORBES, M.A., PH.D., RICHMOND, VA.

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SINCE it is sometimes very convenient to have a nonprotein-nitrogen method for which blood may be readily obtained either from the finger or ear lobe, we have modified a micromethod in use in our laboratory for a number of years for that purpose. These methods are modifications of the well-known Folin and Wu method, but seem to offer several advantages over the same. Not only is the amount of blood filtrate required and the time needed for digestion much less, but also the tendency for precipitation during digestion is greatly reduced. This is the case if the digestion, especially the latter part, is carried on slowly. In some cases, if the nitrogen content is high it is almost impossible to prevent precipitation during digestion. Consequently in these cases, the determination should be repeated using a lesser amount of filtrate.

The procedure used for our regular method is essentially the same as Folin and Wu's method, except that one-fifth the amount of each solution is used and the tubes are graduated at the 10 c.c. instead of the 50 c.c. mark. The procedure is as follows: 1 c.c. of blood filtrate is transferred to a Pyrex test tube, 150 by 15 mm., graduated at 10 c.c., 0.2 c.c. of Folin and Wu digestion mixture added, and digestion carefully carried out in the usual manner. Distilled water is then added to the 10 c.c. mark, the solution mixed, and 3.5 c.c. of Koch's

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\*From the Departments of Biochemistry and Medicine, Medical College of Virginia.  
Received for publication, April 14, 1934.



Nessler solution added. A standard is prepared at the same time, using 2 c.c. of an ammonium sulphate solution (1 c.c. = 0.02 mg. N.), 0.2 c.c. of digestion mixture added, diluted to 10 c.c. and 3.5 c.c. of Nessler solution added.

TABLE I

COMPARATIVE NONPROTEIN-NITROGEN DETERMINATION BY SEVERAL METHODS.  
VALUES IN MG. PER 100 C.C. OF BLOOD

DETERMINATION	FOLIN & WU'S METHOD	MICRO-METHOD	FINGER METHOD
		28.6	29.2
1	27.2	37.8	38.2
2	38.8	35.0	34.8
3	35.2	30.4	31.0
4	30.8	36.0	37.8
5	37.0	34.6	34.0
6	35.8	38.0	38.6
7	38.4	29.8	28.6
8	31.0	34.0	32.8
9	34.0	38.0	40.0
10	----	36.0	36.0
11	----	24.0	24.0
12	----	31.8	31.6
13	----	18.2	18.0
14	----	31.4	30.6
15	----	33.4	34.0
16	----	37.8	37.8
17	----	28.4	30.0
18	----	40.0	40.0
19	----	39.0	39.0
20	----	36.0	35.8
21	----		

In the first 14 the same blood was used for the three determinations, the same filtrate being used for the Folin and Wu and the micromethod. For the remainder, blood was taken by venapuncture in one case and from the finger in the other.

The finger tip method is essentially the same as above, 0.2 c.c. of blood is accurately measured in a special pipette and added to 3.4 c.c. of distilled water in a 15 c.c. centrifuge tube, the pipette being rinsed out several times with the diluting fluid. Two-tenths cubic centimeters of a 10 per cent sodium tungstate solution is then added, followed by 0.2 c.c. of  $\frac{2}{3}$  N. sulphuric acid. After mixing and standing for several minutes, the tube is centrifuged at a moderately high rate of speed. Two cubic centimeters of the supernatant fluid is carefully digested with 0.2 c.c. of digestion mixture and analyzed as described above. If desired, the supernatant liquid may be filtered through a small filter paper after centrifuging, but this is as a rule unnecessary.

#### SUMMARY

A micromethod for the determination of blood nonprotein-nitrogen has been described, which requires only 0.2 c.c. of blood and is consequently suitable for finger tip or ear lobe blood.

NOTE: Suitable 0.2 c.c. pipettes may be obtained from A. S. Aloe Co., St. Louis.

# A NEW METHOD FOR THE DETERMINATION OF THE BLOOD CELL VOLUME\*

SIMON JAY MASON, CINCINNATI, OHIO

IN THE study of red blood corpuscles, a simple method was developed for the determination of their volume, which has an accuracy of  $\pm 0.2$  per cent and which requires only a few drops of blood. The instrument employed is a bulb and calibrated capillary stem made of Pyrex which makes use of the hematocrit principle. It is 12 cm. in length and 1 cm. in diameter. The horizontal and

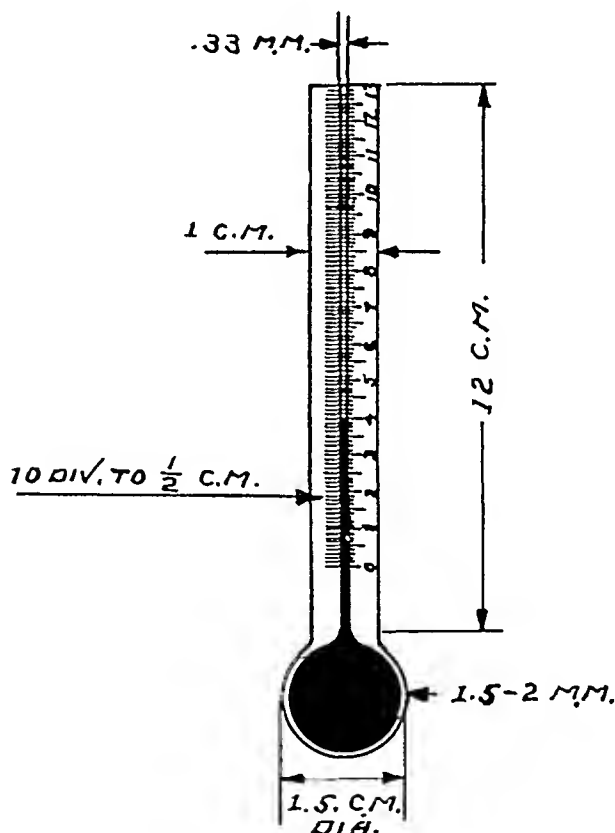


Fig. 1.—Bulb and calibrated tube.

diameters of the bulb are approximately 1.5 cm.; and the thickness of should not be less than 1.5 mm. The bore is 0.33 mm. and accurately ed. The outside markings are spaced to give ten to every half centi- (Fig. 1).

\*From the Kettering Laboratory of Applied Physiology, in the University of Cincinnati. Received for publication, April 9, 1934.

The bulb, filled with mercury, is heated in a small yellow gas flame and when the rising mercury column reaches the level of the tube, sufficient heparinized blood is placed upon the orifice and the bulb immersed in cold water. The contracting mercury draws after it a column of blood which fills the capillary. Excess blood is wiped off and the instrument is placed in a centrifuge cup; in the latter it is centered with a flanged cork, balanced against a cup containing the required amount of sand, and centrifugalized to constant volume. One half hour at 3,800 r.p.m. is sufficient for the purpose. The height of the entire column of the sample and that of the corpuscles is read from the calibration on the outside of the tube (greater accuracy being secured with the aid of a lens), and the percentage of red blood cells per hundred cubic centimeters is calculated.

To remove the blood, the bulb is heated in a blue Bunsen flame until all the mercury is driven out. The stem must not be heated. This leaves the tube

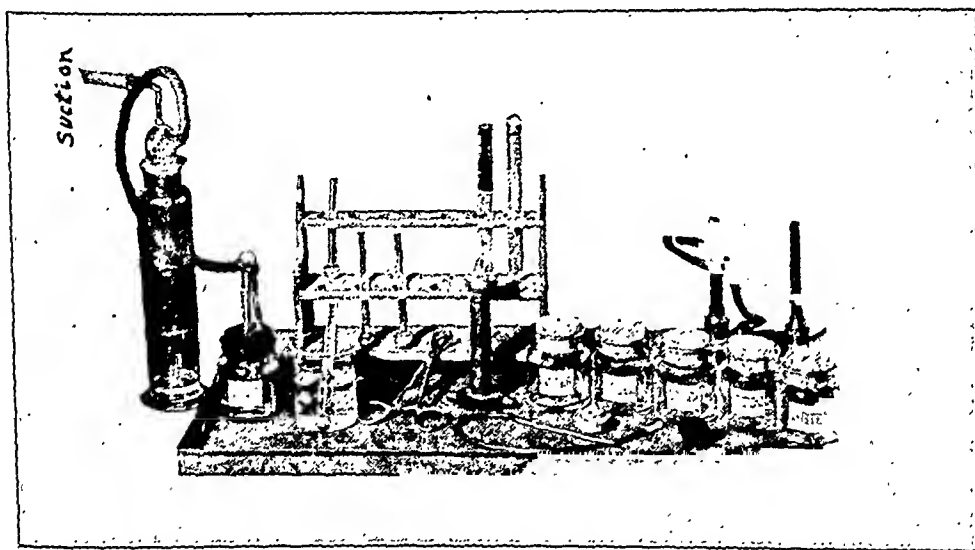


Fig. 2.—Apparatus.

clean and ready for refilling. The instrument can be used several times before any other cleaning is necessary. When this is needed, the empty bulb is heated slightly and inverted into a test tube containing concentrated sulphuric acid. The acid is then drawn up into the cooling bulb. After a few hours the acid may be removed by renewed heating. The outside of the tube is washed with water and inserted, stem down, through one opening of a two-hole rubber stopper; the other opening is occupied by a piece of glass tubing connecting to a strong water jet exhaustor. The rubber stopper is now inserted successively into the neck of each of a series of jars containing, respectively, water, alcohol, ether, acetone; and the suction is so manipulated, together with turning the bottle upside down and righting it, that the bulb and tube are alternately filled with the desired cleaning material and emptied, repeatedly. The instrument is filled with mercury in the same way, the tube and jar being inverted and sustained by a rack. When the tube has been partially filled, the mercury is driven out by heating in

order to completely remove traces of acetone, the presence of which interferes with subsequent control of the mercury column. The bulb and tube are then refilled with mercury, centrifugalized for a minute or two, and the instrument is ready for use. The mercury column should reach about 2 cm. above the bulb. The equipment employed is illustrated in Fig. 2. (Precaution must be taken against the inhalation of vaporized mercury. It is advisable when emptying the bulb to have a jar into which the instrument, inserted in the rubber stopper, is placed, with suction going. The vapors of the mercury are thereby drawn off and condensed. The cleaning of the bulb should be carried out under a well-ventilated hood. The jars and the gas burners should be placed in a low rimmed pan so as to prevent the scattering of the mercury.)

Blood is obtained by drawing it from a freely bleeding finger or ear into a blood cell diluting pipette.<sup>2</sup> Before the blood is drawn, a 2 cm. column of 2 per cent solution of heparin is taken into the pipette stem, after which the pipette is filled to one-quarter or one-half of its volume. The slight dilution introduces only an inconsequential error. The pipette is gently shaken for thorough mixing with the heparin and the first drop of its contents discarded. The determination can be delayed for a while, but better results are obtained by carrying out the determination immediately.

A tenaculum forceps may be used to hold the instrument when the bulb is placed in the blue Bunsen flame for cleaning. When heating for the mercury rise, prior to the introduction of a blood sample, it is held by hand. Care must be taken not to immerse the bulb in water after heating in the blue flame, but breakage does not occur from immersion in water after heating in the yellow flame for a period sufficient to elevate the mercury column to the top of the tube.

During the centrifugation the cover of the centrifuge should be left open, in order to maintain air circulation to prevent an appreciable rise in temperature in the instrument and the sample above that of the room. For the same reason the bulb of the instrument must not be handled. The height of the blood column should be read immediately after centrifugation.

The following are the results (Table I) obtained when determinations were made on two samples of rabbit blood taken simultaneously from the same animal, employing two separate instruments.

TABLE I

RABBIT	% CELLS	RABBIT	% CELLS
13	35.9 35.9	38	27.8 27.4
53	35.74 35.3	95	43.63 43.85
55	33.64 33.8	3	39.1 38.7
225	33.7 33.3	109	40.3 40.7

\*A number of short stemmed pipettes may be blown from small bored soda glass tubing, and the requisite amount of the 2 per cent heparin solution may be dried on the inside of these pipettes.

The following are the results (Table II) of a series of determinations made successively on the same animal, using separate instruments.

TABLE II

RABBIT	% CELLS	TIME	RABBIT	% CELLS	TIME
41	35.6	0 min.	438	35.6	0 min.
	35.2	20 min.		35.7	15 min.
	35.0	1 hr. 20 min.	59	33.5	0 min.
	35.3	2 hr. 20 min.		33.8	5 min.
	35.3	3 hr. 20 min.		33.9	25 min.
83	35.0	4 hr. 20 min.		33.6	45 min.
				33.0	1 hr. 0 min.
	39.7	0 min.	113	39.6	0 min.
	39.3	5 min.		39.8	35 min.
	39.6	20 min.		40.1	50 min.
112	39.1	40 min.		40.0	1 hr. 20 min.
	39.1	1 hr. 0 min.	57	39.1	0 min.
	39.4	1 hr. 35 min.		39.2	37 min.
	40.9	0 min.			
	40.8	20 min.			

#### SUMMARY

A modification of the hematocrit method for the determination of blood cell volume has been devised, which has an accuracy of  $\pm 0.2$  per cent, and which requires only a few drops of blood.

### SIMPLE APPARATUS FOR KEEPING CITRATED BLOOD WARM DURING INJECTION\*

LEONARD HEDDICK, WHITTIER, CALIF.

THE simple and inexpensive apparatus here illustrated can be easily made out of material found in any laboratory. Citrated blood or other intravenous solutions can be readily kept warm by simply filling up the outer jar with warm water. As the temperature cools it can be drained out from the outlet provided in the bottom of the jar and more warm water added. The rate of flow of the blood is clearly visible, being greatly magnified. The apparatus is readily portable, does not need to be sterilized and can be used wherever warm water is available, as nothing else is required.

The warming chamber consists of an ordinary glass battery or museum specimen jar seven inches in diameter and seven inches deep. In the center of the bottom of the jar a circular hole is cut  $1\frac{1}{2}$  inches in diameter. A similar hole, 1 inch in diameter, is also cut out near the side. In this smaller hole a rubber stopper is fitted, through which is thrust a piece of glass tubing. A small piece of rubber tubing with pinch cock attached is fastened on the lower end to serve as the outlet for the cooled water.

\*From the Murphy Memorial Hospital.  
Received for publication, April 18, 1934.

A No. 8 rubber stopper and rubber tubing are slipped on to the lower end of a long tipped Kelly infusion jar. This part of the apparatus is kept wrapped and sterilized. When assembled the infusion jar will rest firmly upright upon the rubber stopper and require no additional support.

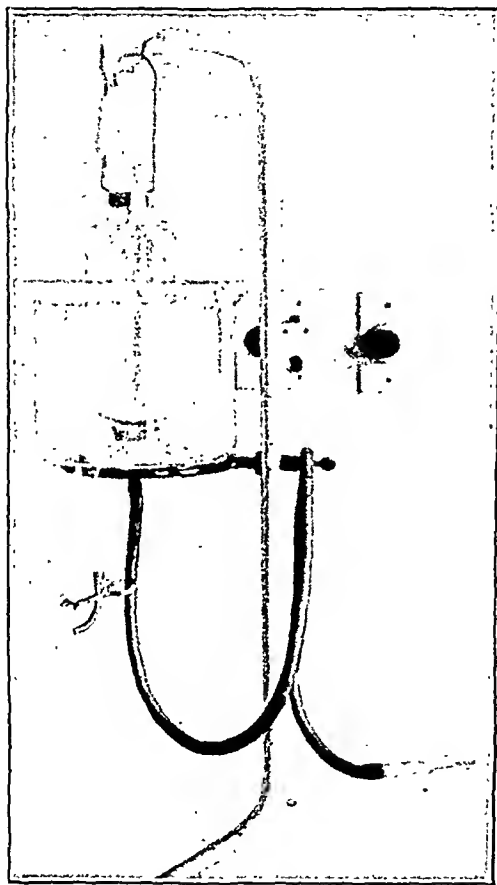


Fig. 1.

A small quantity of saline is used to start the injection, the apparatus is set on the iron ring holder, and the blood to be injected poured over the saline. The warming jar is then filled with warm water of the desired temperature.

The support on the standard is an iron ring with a one-inch segment cut out to allow the tubing to hang straight down.

This simple warming apparatus has been in use by the attending physicians of the above hospital for the past five years and has proved entirely satisfactory.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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## **INFLAMMATION: Cytological Picture of an Inflammatory Exudate in Relation to Its Hydrogen Ion Concentration, Menkin, V. Am. J. Path. 10: 193, 1934.**

A pleural inflammatory exudate, in the majority of instances, develops a rise in its hydrogen ion concentration concomitantly with the progress of the inflammatory reaction.

When the pH of the exudate is alkaline the percentage of polymorphonuclears at the site of inflammation exceeds that of the mononuclear phagocytic cells.

When the pH of the exudate is approximately neutral the percentage of polymorphonuclear cells tends to approach that of the mononuclear phagocytes.

When the pH of the exudate is definitely acid large numbers of polymorphonuclear cells are found degenerated. The percentage of relatively normal appearing polymorphonuclear leucocytes is found considerably lower than that of the mononuclear phagocytes.

In some cases the pH of the exudate remains alkaline throughout the period of an acute pleural inflammation. In these instances the percentage of polymorphonuclears invariably exceeds that of the mononuclears.

By measuring the hydrogen ion concentration of an inflammatory exudate the character of the cytologic picture can be predicted with a fair degree of certainty. Likewise the converse follows.

Evidence has been obtained to show that the development of a local acidosis in an area of inflammation precedes at times the changes occurring in the differential leucocyte formula of the exudate. In such cases, however, the cytologic changes ultimately follow the development of the acid reaction.

The observations reported suggest that the differential leucocyte formula in an area of acute inflammation is a function of the hydrogen ion concentration of the exudate. The cytologic picture in an inflamed area seems to be conditioned by the pH of the exudate surrounding the injured tissue. The present study indicates that the developing local acidosis as the inflammatory reaction progresses can adequately account for the shift in infiltration from polymorphonuclear leucocytes to mononuclear phagocytes at the site of inflammation.

## **DIPHTHERIA TOXOID, Evaluation of, and Methods of Immunization, Monroe, J. D., and Volk, V. K. Am. J. Pub. Health 24: 342, 1934.**

After considerable experience with toxin-antitoxin and toxoid, the authors are of the opinion that toxoid is preferable because it produces a higher degree of immunity in a shorter time. As a result of a previous study of 1,282 children, four injections of toxin-antitoxin gave 74 per cent of Schick negative children at the end of one year, while two injections of toxoid with alum gave 88.8 per cent Schick negative at the end of two months and 94.3 per cent at the end of one year.

Toxoid of 7½ to 10 antigenic potency was preferable to the preparation of concentrated toxoid used by the authors.

Toxoid with the addition of 0.2 per cent of alum is a preferable preparation because it produces a higher degree of immunity as determined by the Schick test in a shorter time than any other used in this study.

It appears that three 1 c.c. injections of toxoid with alum would be ideal, yet for practical application two injections are sufficient.

A 1 c.c. dose produces a slightly higher percentage of reactions than a ½ c.c. dose but produces a higher degree of immunity.

The slightly higher frequency of local reactions from toxoid with alum should not be an objection because the reaction is mild and short. Moderate general reactions occurred in 7 per cent of cases.

In order to reduce the number of visits to a minimum, injection of toxoid and the Schick test at the same visit is suggested for children over five years of age whenever there is no history of previous immunization.

**GRANULOPENIA, Following the Use of Barbiturates and Amydopyrine, Randall, C. L. J. A. M. A. 102: 1137, 1934.**

Granulopenia should not be regarded as a primary type of blood dyscrasia until all possible etiologic factors have been eliminated.

An apparent increase in the occurrence of granulopenia may be related to the widespread use of drugs containing a benzene derivative.

A woman, aged twenty-five, developed an acute and alarming leucopenia following the use of barbiturates and amidopyrine for the relief of a simple headache.

The benzene chain contained in both drugs is possibly responsible for the severe reaction and the disappearance of the granulocytes from the blood.

A number of similar cases have been reported recently.

A difference may be expected in the reaction to benzene quickly absorbed from the alimentary tract, as compared to the type of benzene poisoning that may result from the prolonged, slow absorption that occurs in industrial exposure.

Individuals are known to vary greatly in their susceptibility to benzene. Granulopenia following the use of barbiturates and amidopyrine may therefore occur only in those individuals who are unusually susceptible to the benzene chain.

**ANTIGEN, Note on Acetone Insoluble Lipoids in Relation to the Wassermann Reaction, Kolmer, J. A., and Richter, C. E. Am. J. Clin. Path. 4: 235, 1934.**

It is possible to increase the specific antigenic sensitiveness of alcoholic extracts of beef heart by increasing the amounts of alcohol soluble but acetone insoluble lipoids.

Such extracts sensitized with no more than 0.2 per cent cholesterol possess a very high degree of antigenic sensitiveness with no increase of nonspecific or anticomplementary properties.

Antigens of this kind permit the use of larger amounts in conducting the Wassermann test with an increase of specific sensitiveness for syphilis antibody.

**BLOOD SEDIMENTATION, in Tuberculous Children, Reilly, W. A. Am. Rev. Tuberc. 29: 220, 1934.**

This study comprised 104 tests carried out on 36 tuberculous girls between the ages of eight months and fourteen years, from October, 1930, to March, 1931. Total white blood cell counts on fixed smears with Wright's stain and on supernatally stained smears (the latter for observing monocytes) were done at times for correlation and comparison.

The hilar lesion in its active stages, presumably in the hilar lymph nodes, was generally accompanied by a shortened sedimentation time. This was true in 22 of 23 such patients. The most rapid times, however, occurred in the blood of those children with more active lesions than are usually found in the hilar lymph nodes. It was thus when cavitation of the lung (2 patients) or miliary tuberculosis existed. Almost as rapid times were observed in patients with other disseminations, such as developing Pott's disease (2 patients) and the so-called allergic phenomena, phlyctenular keratoconjunctivitis (2 patients) and pleural effusions (2 patients). Occasionally, however, almost as short sedimentation times were found in the blood of children with hilar lesions.

Of the 33 patients considered clinically active, this was confirmed in 31 patients by a shorter sedimentation time. The remaining two patients had normal or longer times.



Compared or correlated with other laboratory tests for tuberculous activity, the sedimentation time indicated pathological effects much more frequently. Taking 6,000 to 12,000 as the normal range of total white blood cells, there were only rare increases in these children, whereas the Linzenmeier test always gave a rapid sedimentation time.

It is maintained that monocytes frequently increase in the blood of patients with tuberculous activity (3). The sedimentation time shows a consistent shortening. In 20 comparisons, the ratio of monocytes to lymphocytes did not indicate activity, whereas the sedimentation time was rapid in all 20 of these. Only once in 40 comparisons made on 23 patients did the ratio indicate activity and the sedimentation time fail to do so.

The Linzenmeier red blood cell sedimentation time done on 36 tuberculous girls 104 times was found to be a more reliable procedure in the prognosis than in the diagnosis of tuberculous activity. It is also of presumptive aid in diagnosing activity. The author believes it has an advantage over the usual signs, symptoms, and laboratory procedures in determining activity.

**TULAREMIA, The Isolation from the Rocky Mountain Wood Tick of Strains of *B. tularensis*, etc., Davis, G. E., Philip, C. R., and Parker, R. R. Am. J. Pub. Health 19: 449, 1934.**

Three strains of *B. tularensis* of low virulence for rabbits and guinea pigs have been isolated from *Dermacentor andersoni*. This is in marked contrast to numerous other isolations from this tick.

The occurrence in nature of strains of *B. tularensis* of different degrees of virulence is indicated.

When making tests to determine the relative virulence of strains, it is believed that the use of both guinea pigs and domestic rabbits will prove advantageous.

**ENTERITIS, Acute, in Infants and Young Children: Clinical Study in Forty-Six Patients: Necropsy Studies, Cooper, M. L., Keller, H. M., and Johnson, B. Am. J. Dis. Child. 47: 596, 1934.**

Five of the children were studied at necropsy. All showed gross and microscopic pathologic changes in the gastroenteric tract. Cultures of intestinal contents at various levels of the intestinal tract were made in the usual manner to avoid contamination; i.e., a sterile cotton swab was inserted through an incision in the intestinal wall made with sterile scissors in an area sterilized by application of a hot spatula. Postmortem cultures were made immediately after obtaining the material. This material was cultured and studied in the way described for cultures of stools.

Streptococci (*Streptococcus micro-apoikia*) were isolated from the contents of the involved intestine in four of these five patients and from the blood stream in the fifth. Four of these 5 cultures of streptococci were used for injection into animals, and all 4 cultures produced the characteristic pathologic changes in the animals' intestinal tract and were subsequently recovered from the animals.

A brief summary of the necropsy studies shows that of five patients studied at necropsy all had gross and microscopic pathologic changes in the intestinal tract and that *Streptococcus micro-apoikia enteritidis* was isolated from the intestinal tracts of 4 patients and from the blood of the fifth. Of 6 monkeys which received injections of this streptococcus 5 died, and these showed pathologic changes in the intestinal tract; streptococci were recovered from the intestinal tract of each. Ninety rabbits were given injections and 70 per cent of these died. Ninety-two per cent of these animals studied at necropsy showed characteristic pathologic changes in the intestinal tract, and cultures of the contents of the intestinal tract of animals at the time of necropsy showed the presence of *Streptococcus micro-apoikia enteritidis* in 80 per cent.

*Streptococcus micro-apoikia* was isolated from 55 per cent of 51 stools from 46 patients. The intestinal tracts of 5 patients cultured at necropsy contained these streptococci.

No streptococci similar to *Streptococcus micro-apoikia* were recovered in the control experiments in which seventy specimens of stools from 49 patients who had no intestinal infection were cultured.

Rabbits which received injections of the original mixed culture of stools or intestinal contents died; typical lesions were found in their intestinal tracts, and from these areas *Streptococcus micro-apoikia enteritidis* were recovered.

Rabbits which received injections of pure cultures of streptococci from the blood, post-mortem material or stool of the patients, from a suspension of a patient's stool, from the peritoneal washings of mice which had received injections or from rabbits that had been given injections showed the same typical pathologic changes in their intestinal tracts, and from these areas the streptococci were recovered.

Organisms from rabbits which received injections of somewhat similar green-producing streptococci isolated from stools and other sources in patients free from any enteric infection were but slightly virulent, and in only a few instances did cultures produce intestinal lesions. These cultures were from the throats of two patients with acute pharyngitis and from the mastoid cells of two other patients with acute mastoiditis.

*Streptococcus micro-apoikia enteritidis* was not found in the intestinal tracts of normal rabbits studied at necropsy.

Convalescent rabbit serum protected rabbits against *Streptococcus micro-apoikia enteritidis*.

Rabbits remained normal when given injections of Berkefeld filtrates of cultures of streptococci recovered from patients with acute enteritis, of the blood purulent contents of the involved intestine or of peritoneal washings of mice that had been given injections.

Six macacus rhesus monkeys were given injections of streptococci recovered from patients with acute enteritis. The 6 monkeys became ill, and 5 died. All five showed typical lesions in the small intestines, and the streptococci were isolated from the contents of these areas. These reisolated streptococci produced the same lesions in the intestinal tracts of rabbits. Cultures of stools of the monkeys before injection did not contain these streptococci, but the organisms were present in cultures of the diarrheal stools after injection.

Generally, the first third of the small intestine of the rabbit or the major portion of the small intestine of the monkey appeared deeply injected. The lymphoid tissue in the wall of the involved portion of intestine was enlarged and purplish red. The contents of this portion of the intestine were blood-streaked, thick and purulent. The nodes in the mesentery, especially those adjacent to the intestine, were enlarged and deeply purple.

Microscopic study of sections of the small intestine revealed marked congestion in the mucosa, a polymorphonuclear and monocyte reaction in the mucosa and the submucosa and diplococci in the mucosa. Sections of mesenteric nodes revealed acute lymphadenitis and prominent germinal follicles with reticulo-endothelial hyperplasia in their centers. Sections of the spleen showed severe acute splenitis, congestion of the pulp with some hemorrhage and necrosis of the malpighian corpuscles and a polymorphonuclear reaction. The liver showed vacuolation of many of its cell nuclei. The kidneys gave the picture of a toxic nephrosis. Other tissues were normal.

#### **BACTERIOPHAGE, Specific Treatment of Septic Infections, Particularly With the Aid of, Mac Neal, W. J. Am. J. M. Sc. 187: 623, 1934.**

The early specific diagnosis of infectious disease by immediate application of the appropriate bacteriologic technic is strongly urged.

Specific diagnosis facilitates proper care of the patient and isolation of the specific microbe makes possible the intelligent selection of specific serum or specific bacteriophage.

The appropriate application of antistreptococcus serum or of bacteriophages against the staphylococcus or the colon bacillus requires intimate collaboration of clinician and laboratory worker. The use of these agents is recommended as beneficial to some patients suffering from infection with the respective microbes.

# NEUTROPENIAS, Effect of Leucocytic Cream Injections in the Treatment of, Strumia, M. M. Am. J. M. Sc. 187: 527, 1934.

Evidence is presented that injections of leucocytic cream intramuscularly in severe neutropenias is followed in most cases within one to four days, usually forty-eight hours, by an increase of mature granulocytic cells in circulation, along with a considerable clinical improvement.

The preparation of the leucocytic cream is as follows:

Withdraw the blood from the vein of the donor into citrate solution not over 150 c.c. Divide the blood in large tubes and centrifuge at high speed for thirty minutes. Carefully withdraw the clear supernatant plasma, part of which is kept for the preparation of leucocytic emulsion later. With a large mouth pipette and a rubber bulb remove carefully the buffy layer of leucocytes which has formed on the surface of the packed red cells. Do that for each tube and transfer the material to a Babcock cream tube. Add to this enough of the packed red cells to bring up well in the neck the surface of the fluid. Centrifuge again for twenty minutes at high speed. The leucocytes will now rise to the top. There are usually three well-defined layers: (1) clear plasma; (2) suspension of leucocytes and platelets; (3) leucocytes mixed with some red cells. This last layer has been found to be richer in polys, while the topmost layer contains more lymphocytes. Draw off with a large mouth capillary pipette this material, removing also the top layer of the packed erythrocytes. Suspend this material with plasma so as to make for each 100 c.c. of blood used from 5 to 10 c.c. of suspension. The material is used intramuscularly. As a rule, with high speed of the centrifuge the volume of the packed leucocytes is 1.6 per cent of the amount of blood used, and the suspension contains about  $\frac{2}{3}$  of the leucocytes present in the blood used. In smears of the suspension, the leucocytes appear very well preserved. It is desirable to check with a smear, or better, a cell count, that the suspension prepared is adequately rich in leucocytes. It is essential to separate the leucocytes from the blood as early as possible after the withdrawal of the blood from the donor. If for some reason the blood must be kept overnight, twice the amount of citrate should be used. The leucocytes thus prepared remain well preserved for a period of seven days at least, although the author never used them after five. Of course strict aseptic technique must be employed. Another important point is to remove as much of the plasma as possible after the first centrifugation and to have the topmost layer of the material transferred in the Babcock cream bottle as high as possible in the neck. If for some reason (deficiency of red cells) this is not possible, the surface of the fluid may be brought to the proper point by introducing in the Babcock cream bottle sterile glass beads. If the material necessary is ready at hand the whole procedure for 500 c.c. of blood can easily be accomplished in two hours or a little less.

The citrate solution, as anticoagulant, is prepared by dissolving 20 gm. of sodium citrate in enough sodium chlorid solution to make 100 c.c. The sodium chlorid solution percentage is 0.85. This solution contains for each c.c. 200 mg. of sodium citrate, which is sufficient to prevent coagulation of 50 c.c. of blood.

## SICKLE CELL ANEMIA, Negative Results in the Treatment of, Diggs, L. W. Am. J. M. Sc. 187: 521, 1934.

Seven negro patients with sickle-cell anemia were treated with one or more of the following antianemic medications: whole liver, liver extract by mouth, liver extract subcutaneously, desiccated hog's stomach, bone marrow extract, iron, and transfusion. In 6 cases the erythrocyte, hemoglobin and reticulocyte values, and the clinical condition following therapy did not significantly change. In one negro child with the sickle-cell trait, observed during an initial anemic crisis associated with bronchopneumonia, clinical improvement and improvement of the anemia followed transfusion and the administration of liver and iron.

There is a dearth of information concerning the natural course of sickle-cell anemia in all its varied manifestations, and the possibility of spontaneous remissions must be considered in the evaluation of favorable therapeutic responses. The effectiveness of any form of therapy in the treatment of sickle-cell anemia remains to be proved.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

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### Electrocardiography\*

THE student and practitioner of medicine in these days, if he is to be successful in his endeavor to understand the aspects and manifestations of disease, must be prepared to familiarize himself with the many innovations confronting him.

While the stethoscope and the percussing finger are still essential to the study of diseases of the heart, even the skilled physician must take cognizance of the rapid advances which have been made in the physiological field of cardiology, the practical application of which is found in the electrocardiogram.

No one will dissent from the author's prefatory statement that: "There is need for a treatise on electrocardiography, for use by the general practitioner, the medical student, and the specialist exclusive of the cardiologist, that they may learn the value of the electrocardiogram in the diagnosis of cardiac disease and learn to correlate the electrocardiographic findings with their clinical data. To meet that need this volume has been written."

The volume begins with a brief but excellent summary of the clinical concepts of heart disease and arrhythmias, followed by a discussion of the conduction system. The principles and mechanism of the electrocardiograph are then clearly explained followed by a clear-cut discussion of the electrocardiogram. The remaining chapters are devoted to the clinical applications of electrocardiography illustrated by many graphs and their interpretation.

It is certain that to those who read this book with care electrocardiography will cease to be an esoteric subject and become a useful clinical aid.

There are few axioms more pertinent or which should be more often emphasized than that which closes the discussion of the clinical concepts of disease; namely: that whenever the cardiovascular system of an individual is under consideration there are four questions to be answered: (1) What caused the cardiac disease? (2) What is the structural lesion? (3) How is the physiology changed? and (4) What are his limitations?

This book may be recommended as presenting a very clear and practical discussion.

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### A Textbook of Histology†

THAT this textbook intended primarily for medical students has passed through six editions in eight years indicates its usefulness.

The present edition has been thoroughly revised and to a large extent rearranged and rewritten, particularly in the sections concerning the blood, the reticulo-endothelial system, the endocrine tissues, striped muscle, neuroglia, nervous tissue, the reproductive systems, and the lymphoid organs.

The final chapter (22 pages) presents an excellent discussion of histologic technique.

Both as a text for the student and as a work of reference this book is of use and value and for these purposes it may be thoroughly recommended.

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\*Electrocardiography. By C. C. Maher, M.D., Assistant Professor of Medicine, Northwestern University. Cloth, pp. 250, 39 figures. William Wood and Co., Baltimore, Md.

†Textbook of Histology. By Harvey E. Jordan, Ph.D., Professor of Histology and Embryology, University of Virginia. Edition 6, cloth, pp. 738, 609 figures. D. Appleton-Century Co., New York.

THE volume presents not only a comprehensive discussion of toxicology in general but incorporates an extensive discussion of industrial poisoning and a section on the prevention and compensation of industrial poisoning, by F. Koelsch, Director of the Bavarian Institute for Industrial Medicine, and a further section on the detection of poisoning, post-mortem, by K. Meixner, Professor of Forensic Medicine, Innsbruck.

The references include material appearing as late as 1932, the American literature not being neglected although naturally the majority of the references are from European sources.

The volume may be recommended as a useful and practical addition to the reference library on this subject.

WHILE primarily intended for the medical student, Folin's Manual for many years has been a useful reference text for the clinical laboratory and needs, therefore, no introduction.

The supplement, containing a detailed description of the methods used in the examination of urine and blood, will be of particular interest to the clinical laboratory worker because it presents important modifications of technic as well as new and hitherto unpublished data.

These newer methods, yielding results somewhat at variance with older technics, are of importance in this respect and workers in this field must be familiar with them.

As before, this new edition of the manual will occupy the same distinguished position as its predecessors and may be highly recommended without reserve.

\*Clinical Toxicology: " " " " the Diagnosis and Treatment of Poisoning. By Erich Leschke, Professor o. University of Berlin. Translated by C. P. Stewart, Lecturer in General University of Edinburgh and O. Durrer, Research Assistant to Professor Wieland, Munich. Cloth, pp. 346, 25 figures. William Wood & Co., Baltimore, Md.

†A Laboratory Manual of Biological Chemistry. By Otto Folin, Professor of Biological Chemistry, Harvard University. Edition 5. Cloth, pp. 368. D. Appleton-Century Co., New York.

# The Journal of Laboratory and Clinical Medicine

Vol. 20

DECEMBER, 1934

No. 3

Editor: WARREN T. VAUGHAN, M.D.  
Richmond, Va.

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## EDITORIALS

### Bacon Was Right

**B**UFFETED as he is by the battledore and shuttlecock of opposing demands, the doctor's is surely one of the most paradoxical of all existences. The responsibilities thrust upon him by the nature of his duties certainly deserve, if they do not compel, opportunities for quiet, sober consideration—and yet his decisions, however momentous, must often be made upon the spur of the moment and from such facts as can be gathered rapidly under stress.

Although many an obscure and puzzling case really *demands* concentrated thought, the doctor's leisure, such as it is, is at the mercy of every hurried emergency. Many a battle against disease without doubt were best waged on a plan evolved after a council of war, so to speak, with leaders, experts, and authorities marshalled from the doctor's bookshelf, but no psychologist is required to determine the reaction of the patient requested to wait the formulation of advice until authorities had been consulted!

So, it would seem, the doctor's reading must often be done largely "on the run," which, perhaps, accounts for the somewhat sorry appearance of some

professional "libraries," too many of the volumes weak-kneed and broken-backed with age and, alas, somewhat frowsy from neglect.

The most common reply to this stricture—and one may rest assured that it is made more often than it is spoken, and has certainly occurred more than once to the intelligent patient—is that it is almost impossible to keep up with the flood of medical literature. To strengthen the alibi further it is often said—and with some truth—that medical literature is ephemeral literature; the up-to-the-minute "systems" of today are behind the times tomorrow; and the facts of one edition are the archaisms of the next.

To a large extent this is true, for while no one can deny that the two basic elements upon which the practice of medicine is founded—human beings and disease—are of undoubted antiquity, the angle of approach and the viewpoint from which they must be considered is constantly changing and undergoing modification, readjustment, and realignment in the light of changing concepts.

The problem in essence is to understand the mechanism involved and the results produced by the reaction of one variable, the individual patient, to another variable, the stimuli exerted by disease. And the understanding of either and both is constantly being revised, clarified, and augmented by a reconsideration of old concepts in the light of new facts, and by the formulation of new concepts arising from new observations which may or may not be justified, confirmed, or discarded by the cumulative evidence evolving from time and extended trial.

Thus it is that the ponderous, many-volumed sets of yesteryear are giving way to the more flexible publications capable of revision, and to the monographs which present in a single volume the current status of a single, circumscribed problem.

Even yet, however, there are difficulties, for the court of last resort is recorded experience. The experiments, the observations of today, must run the gauntlet of critical repetition and must stand or fall upon cumulative experience. No matter how promising they appear to be they cannot take their place in system, text, or monograph until a reasonable basis for their acceptance exists, and such reasonable basis arises from the recorded observations which accumulate in the various medical journals, each covering a more or less restricted field.

The medical textbook of tomorrow is, after all, really a digested critical summary of the medical literature as reported in the medical journals of which the number and variety groweth apace. Verily, the physician of good intention struggles in a flood of print—and who should say offhand which of it will join the steady stream of knowledge and which will pass as spray blown into nothingness by the winds of time and trial?

So it is often heard that there isn't time to read the medical journals; that they are too scientific and report too much scientific work (!); that they are not "practical" enough, and so on—and too often it is true that those who protest the loudest might benefit the most.

It would be impossible, of course, for anyone to read all the journals nor, indeed, is it worth while to read always all and every page of those he does

open—for no man can hope to know all of medicine as it is today. Every man must have some compelling interest, and there are few times, indeed, when there will not be something he may read with interest and profit.

Fortunately, in the abstract journals, there are available also concentrated surveys of the medical literature of the world which present briefly and succinctly the meat of lengthy contributions, the facts reduced to scientific syllogisms as it were, indicating almost laconically the basis for the ideas advanced. It is almost impossible to make this literary menu all-inclusive and keep within reasonable bounds; it must represent, therefore, the gist of the news.

Just what should be the basis for inclusion of an article? Some would have it entirely “practical”—but just what shall this mean, for who can tell in advance which research, which reported observation is practical and which abstract in the long run?

There can be no doubt of the practical value of the use of liver in pernicious anemia, yet this only came to pass because Whipple first studied the results of secondary anemia in dogs and a long time elapsed before the application of these purely abstract and scientific studies to the treatment of pernicious anemia in the human being was made by Minot and Murphy.

d’Herelle’s studies in their inception were surely rather purely “scientific” and apparently only of somewhat academic interest, yet who can foretell the eventual relation of bacteriophage to the treatment of disease?

On the other hand, the “practical” observation of Dr. So-and-So upon the marvelous results of this or that method of treatment may be merely a question of conclusions based upon “one consecutive case”; or the result of imperfectly analyzed facts or imperfect or incomplete investigations.

It is not improbable that the main purpose of what sometimes passes for medical “literature” is to boost sales and that often it is read because it is so dogmatic and so certain and requires of the reader nothing but unquestioning acceptance of its statements.

The real purpose of scientific reading is twofold: to acquire information and to stimulate thought.

The periodical appearance of new “cures” for cancer in the public press is familiar to everyone—and how can the doctor answer the questions of his patients on such matters unless he has some knowledge of the present status of cancer research?

Unless he is to stagnate, content to fall behind and finally to become really ignorant of the things he should know, the doctor *must* read.

A library is only an asset when it is consulted and journals are of little use when stacked in dusty piles with the wrappers unbroken. If he must “read on the run” then let him read the abstract journals, and be it remembered that, whether their contents appear always and immediately “practical” or sometimes apparently merely abstractly scientific, they are always informative, always additions to one’s general knowledge, and many times quite useful to have read. At least they will always furnish something to think about.



Bacon was right when he said: "Reading maketh a full man; conferenee a ready man; and writing an exaet man; and, therefore, if a man write little he need have a great memory; if he confer little he need have a present wit; and if he read little, he had need have much cunning, to seem to know that he doth not."

—R. A. K.

### Postoperative Wound Infections

WHILE the development of infection in originally clean surgical wounds is no longer the bugbear it was before the days of Pasteur and Lister, its occurrence is not yet a *rara avis*, and constitutes one of the most annoying and distressing complications of surgery.

Not only is it the immediate cause of prolongation of hospitalization and hence of increased expense, not to mention the suffering, anxiety, and loss of confidence in the surgeon with which it is often associated, but should the surgeon be so unfortunate as to encounter a virulent postoperative infection resulting in the death of the patient, the catastrophe is one almost impossible to explain to the satisfaction of all concerned.

When postoperative infections occur, therefore, and especially when they are more than very occasional in incidence, they are at once the subject of concern and discussion and the laboratory is often requested to inaugurate a sometimes extensive series of investigations.

It is, of course, quite natural and human under these circumstances to regard the operating room force with a questioning eye; to inquire as to the methods of sterilization in force; and to discuss the advisability of an extended bacteriologic survey. But the consensus of experience has shown that the first and essential factor in the survey is not the survey and study of minutia, but the survey of the problem as a whole.

In other words, for the successful management of such a problem it is essential to know, first of all, not merely that infection is present, but to know its *nature*. The first thing to be examined bacteriologically should be the wound. While this should be the invariable routine, it is especially important and may often be the determining factor, when postoperative infections occur in series, whether in the same service, in the same ward, or in a similar series of cases.

Without some definite plan of campaign the search is otherwise haphazard and even almost hopeless, for the possible avenues of infection are well-nigh legion.

The practical impossibility of sterilization of the skin throughout its entire thickness is well known; the metastatic invasion of surgical wounds from distant or cryptic foci is not unknown; and many accidental and even bizarre avenues may be thought of and even illustrated by experience.

But it is not the possibilities which should first be studied but the *probabilities*, and what the preeminent probabilities are can only be estimated when fundamental data are first collected.

It is, as a rule, of little use to begin with extensive culturing of operating room supplies, for it is rare indeed, where dressings, instruments, or solutions are sterilized with intelligence and understanding of the underlying principles that these are at fault, and commercial products are so safeguarded and controlled nowadays that infection from this source is practically unknown.

The place to begin is hence the wound. When infection occurs in series in clean appendectomies and the organisms recovered are predominantly of the colon group, the inferential probability is that their implantation occurred at the time of operation. When the organisms are predominantly of the *Streptococcus* group, the possibility arises that one or more of these concerned in the operations are carriers of this group; whereas, should the *Staphylococci* be in the main the offending organism, attention is directed toward the skin as a probable source of invasion.

When the nature of the infection is known the trend of the investigation becomes somewhat more obvious and, just as in the determination of the potability of water the survey of the source is of equal and sometimes even more importance than its bacteriologic study, so here the next step should be the survey of the operations. For in the vast majority of instances the underlying factor of paramount importance in postoperative wound infections is technique, and in its study keen observation is all that is required.

It is natural for surgeons to disclaim responsibility for infection but nevertheless, when it occurs, it is upon themselves and their methods that they should first focus attention. It is neither fair, just, nor invariably correct to place the burden at once upon the shoulders of the long-suffering assistant. If he be at fault—as sometimes he may be—the responsibility is still the surgeon's, not only for not training him properly by precept and example, but also for not detecting the error when it occurred.

The leaking of skin organisms from the sebaceous glands through a punctured glove is too obvious a source of infection to be overlooked, although this source is occasionally sometimes neglected; and the preoperative preparation of the skin is seldom at fault per se.

It is too often forgotten that bacterial invasion alone is not necessarily followed by infection; the tissues must be so prepared for infection as to permit the growth and reproduction of bacteria subsequent to invasion without which infection does not occur. The other factors often of determining importance, such as the numerical proportion of the bacterial invasion, etc., are, after all, of contributory importance.

In the survey of technique, therefore, attention should be directed to other factors of even greater importance.

Has anything occurred which would produce or contribute to devitalization of the tissues and so render them more liable to bacterial invasion? Has the operator or his assistant an "iron" or a "velvet" hand? Are the tissues manipulated *gently* or is pulling and tugging the order of the day? Are the gauze packs used to wall off the hyperemic and inflamed appendix, on removal dragged across the wound edges, in forgetfulness that if the intact intestinal wall is not impermeable to bacteria the diseased intestinal wall is still less so?

Was the wound dry when closed, or in the endeavor to make it so were relatively large masses of abdominal fat tied off with the vessels, later to necrose and form a fertile field for the skin organisms swept out from the relaxed skin of the perspiring patient?

These—and many more—are all of importance and may be determined without culture flasks or tubes. That they should be studied first of all is often indicated, as already suggested, by the original study of the nature of the infection.

It is well, of course, to survey methods of sterilization and so on, but it is better to survey operating technique for the answer will most often there be found.

Infection can be prevented—but its prevention requires cooperation, teamwork, and, strange as it may seem, not infrequently the legitimate use of a little imagination.

—R. A. K.

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## ITEM

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### The American Association for the Study of Goiter

The American Association for the Study of Goiter again offers the Van Meter Prize Award of \$300 and two honorable mentions for the best essays on the subject of goiter provided they meet the standards of the award committee. The essays should be based on original research work on the subject of goiter, preferably its basic cause. The prize essay or its abridgment is to be presented at the annual meeting of the Association to be held in Salt Lake City, Utah, in June, 1935.

Competing manuscripts should be in the hands of the Corresponding Secretary, W. Blair Mosser, M.D., Kane, Pa., not later than April 1, 1935.

The first prize of \$300 for the 1934 meeting was awarded to M. A. B. Brazier, Ph.D., B.Sc., London, England, for her essay, "The Impedance Angle Test for Thyrotoxicosis."

First honorable mention was awarded Prof. Ugo Cerletti, Genoa, Italy, for his essay, "Three Years of Experimental Research in the Etiology of Endemic Goitre."

Second honorable mention was awarded D. Roy McCullagh, M.D., Cleveland Clinic, Cleveland, Ohio, for his essay, "Studies in Blood Iodine Using a New Chemical Method."

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### Erratum

In the article by Drs. Graham and Randles in the October issue of the Journal, Fig. 1, which appears on page 91, is up-side down.

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THE article entitled "Gauging the Dose of Insulin," in the August issue of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE, was of considerable interest because of the similarity in approach between Dr. Glassberg's calculations and those used by Dr. Henry Larson of our staff during the past year.

Approximately one year ago, Dr. Larson was interested in deducing the maximum dosage of insulin which could be safely given at one time to any diabetic, taking proper consideration of the weight and blood sugar of the patient. The formula derived does not have to be corrected by any factor such as is necessary with that of Dr. Glassberg.

A.  $\left( \frac{\text{BW}}{3} \cdot \frac{\text{BS}}{100} \right)$  where BW represents weight in pounds  
BS represents patient's blood sugar.

By subtracting  $\frac{BW}{3}$  from Formula A, one arrives at the number of grams of excess glucose which have to be metabolized by the insulin administered:

$$B. \left( \frac{BW}{3} \cdot \frac{BS}{100} \right) - \frac{BW}{3}$$

Instead of dividing Formula B by 2, as is suggested in Dr. Glassberg's article, it would be safer to use the factor 2.5, since at its maximum efficiency, 1 unit of insulin can take care of 2.5 grams of glucose.

$$\frac{\left(\frac{\text{BW} \cdot \text{BS}}{300}\right) - \frac{\text{BW}}{3}}{2.5} \text{ equals } \frac{\text{BW} (\text{BS} - 100)}{750} \text{ equals total safe units of insulin.}$$

It is fully realized that the contraindications such as myocardial damage or coronary disease are to be scrupulously observed. As Dr. Glassberg remarks, diabetes cannot be treated merely by formula, since other factors, particularly concurrent infection, decrease the efficiency of insulin and require special consideration. Another point worth mentioning is the fact that insulin shock may result with a blood sugar above 100 mg. per cent; and that apparent comfort may exist with a blood sugar below 100 mg. per cent.

The physician may not wish to reduce the blood sugar to 100 mg. per cent. As a matter of fact, when one is dealing with diabetes of long standing, and the patient presents himself with a very high blood sugar, it might be more expedient to reduce it to 200 or 150 mg. per cent. The chronic diabetic feels more comfortable at such a level. In such a case, if one subtracts 2 or  $1\frac{1}{2}$  times  $\frac{BW}{3}$  respectively, and divides the result by  $2\frac{1}{2}$ , the quotient will represent the number of units of insulin required.

The above formulas are suggested merely as a guide for the use of insulin particularly in cases of real or imminent diabetic coma. In our hands it has proved safe and satisfactory, and the premise is based on the classic work of Peters and Van Slyke.

ATTILIO F. GALASSO, M.D.

OCTOBER 8, 1934  
MORRISTOWN, NEW JERSEY

# *The Journal of Laboratory and Clinical Medicine*

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VOL. 20

JANUARY, 1935

No. 4

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## CLINICAL AND EXPERIMENTAL

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### METEOROLOGICALLY CONDITIONED VARIABILITY OF SEROLOGIC TESTS IN SYPHILIS\*

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EMIL T. HOVERSON, M.A., M.D., KANKAKEE, ILL., AND  
WM. F. PETERSEN, M.D., CHICAGO, ILL.  
WITH THE TECHNICAL ASSISTANCE OF  
MR. DELESTER SACKETT, ELGIN, ILL.

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SINCE the introduction of the Wassermann test, the serologic diagnosis of syphilis has become a field that has attracted the interest of many workers, and as a result of this there is a voluminous literature on the subject. One point which this mass of work has served to have emphasized is the fact that a certain degree of uniformity obtains in all of the various tests for syphilis, so that despite the many modifications of the original Wassermann reaction, and even subsequent variations, there is a close agreement between the serologic findings obtained by the various methods (technical proceedings). Because of this close agreement, it is generally accepted that the results of a blood serum test for syphilis is quite accurate, regardless of the method employed for the determination. Moreover, if the testing is done by well-trained technicians, it is very unlikely that errors in diagnosis will occur except to a very limited extent. As these statements constitute common knowledge it is felt that they call for no further substantiation.

Another belief which is quite generally held is that a change in the serology from positive to negative or vice versa indicates some alteration in the concentration of the syphilitic antibodies. Moreover, since syphilis is regarded as a

\*From the Kankakee State Hospital, and the University of Illinois, The State Psychopathic Laboratory, Department of Pathology, College of Medicine.  
Received for publication, April 16, 1934.

disease process of a chronic progressive character, changes in the serology ought to be gradual, and quite uncommon in the absence of specific treatment. In those cases in which treatment is instituted, the changes in the serology are gradual, sometimes a period of years being required for a reversal from positive to negative findings. One of us (E. T. H.)<sup>1</sup> has elsewhere reported the occurrence of such similar changes in untreated syphilis, but in these instances lengthy periods of time had elapsed between the recording of the first positive test for syphilis, and the final negative test.

The purpose of the present study was to determine if reversals of the serology occurred over long or short periods of time. The results of the study indicate that in certain cases there occurred marked changes in the serology over short periods of time, measured in terms of days. As far as it has been possible to determine, no similar study has appeared in the available literature. True, there are many reports of a single series of cases, but in these the object has been a comparison between methods to establish correlation between different methods. Also comparisons have been made over lengthy periods, and following antisyphilitic treatment.

As is well known, definite variations are found in the blood serology in certain cases of syphilis as a sequel to treatment. Thus in a group of patients having strongly positive serologic evidence of syphilis before treatment, some will show a lessening of the strength of the reaction, and it will be recorded in accordance with common usage as three-plus, two-plus, one-plus, and negative. If the group showing these changes is given a rest period, a certain percentage will have a four-plus reaction, while some will be negative, after some months. These observations are common, but they have been made usually on single and not on consecutive blood samples.

Since, as was already stated, the methods of determination are standardized, and carried out by trained technicians, the results are quite accurate, and it follows that if changes are observed, they are due to other factors than those inherent in the determination itself. All the determinations in this work were made using the Wassermann and Kahn tests (Craig modification) on the same sample of blood. Routine qualitative determinations such as are usually made for ordinary work, were carried out on consecutive samples for a definite length of time. All of this technical work was done by Mr. DeLester Sackett. Because of this, all samples were handled in a uniform manner. The same batch of reagents, i.e., antigen, for every test was used, so that changes would be caused by the specimen and not the reagent.

For the purpose of this study, white male patients afflicted with general paralysis in varying stages of the disease were selected. The only requirements were, first, that the patient had showed positive serologic evidence of syphilis on admission to the Kankakee State Hospital; second, that he had received no form of treatment for at least six months prior to this work; third, that the patient was sane and last, but quite important, that the patient

was withdrawn in a uniform manner, on every occasion during the course of the study, between the

hours of 8:30 and 9:30 A.M. The serum was collected on the same day on which the blood was withdrawn, and the next day it was sent to the State Psychopathic Laboratory.

The ages of the patients ranged from thirty to sixty years. All were free from other physical conditions which might affect the serology. Originally ten patients were selected, but as five of these had negative Wassermann and Kahn reactions on the blood sera on each of the first seven determinations, they were excluded from further participation in this study. In their place five other patients were chosen on the basis on which the first ten patients were selected.

In order to demonstrate the results, the fifteen patients are grouped in three classes as follows:

1. Five patients on whom both the blood Wassermann and Kahn reactions were negative.

2. Seven patients who showed rather striking variations in both the Wassermann and Kahn reactions.

3. Three patients who had strongly positive Wassermann and Kahn reactions on all determinations.

#### GROUP I

TABLE I

THIS TABLE INCLUDES BOTH THE WASSERMANN AND KAHN REACTIONS

DATE, 1934	PATIENT IDENTIFICATION NUMBERS				
	I A.B.	II S.L.	III G.B.	IV J.R.	V M.F.
January					
14	Neg.	Neg.	Neg.	Neg.	Neg.
15					
16	Neg.	Neg.	Neg.	Neg.	Neg.
17					
18	Neg.	Neg.	Neg.	Neg.	Neg.
19					
20					
21	Neg.	Neg.	Neg.	Neg.	Neg.
22					
23	Neg.	Neg.	Neg.	Neg.	Neg.
24					
25	Neg.	Neg.	Neg.	Neg.	Neg.
26					
27					
28	Neg.	Neg.	Neg.	Neg.	Neg.

Thus the five patients in this group had consistently negative Wassermann and Kahn reactions on their blood sera in seven determinations, taken in a period of two weeks.

#### GROUP II

In order better to show the variations, the results on the seven patients comprising this group are placed in two tables; the first (Table II) shows the variations in the Wassermann reaction, and the second (Table III), the changes in the Kahn reaction on the same seven patients.

It is believed that the changes shown in Tables II and III of Group II are significant, and are worthy of further study. The extent of the changes themselves is evident, and needs no comment.

TABLE II  
WASSERMANN VARIATIONS

DATE, 1934	VI J.M.	VII M.K.	VIII F.W.	IX F.K.	X G.T.	XI H.E.	XII L.M.
January 14	-	-	+	-	+++		
15							
16	±	-	+	-	-		
17							
18	±	±	-	+	+++		
19							
20							
21	-	-	-	-	-		
22							
23	-	-	-	-	-		
24							
25	-	±	-	-	±		
26							
27							
28	++	-	-	-	-		
29							
30	+	++	++	-	++++	+	++
31							
February 1	+	+	+	-	++++	++++	-
2							
3							
4	-	-	-	-	-	-	-
5							
6	-	-	++	-	+	++++	-
7							
8	±	+++	++	-	±	++++	-
9							
10							
11	+++	-	-	-	++++	++++	-
12							
13	-	-	±	-	-	+	-
14							
15	+	++	++	-	+++	++++	+
16							
17							
18						++++	-
19							
20						++++	++
21							
22						++++	++
23							
24							
25						++++	-
26							
27						++++	+
28							
March 1						++++	-

## GROUP III

The results in this group are placed in a single table (Table IV); the first three figures on the left show the Wassermann, and the remaining three columns show the Kahn variations.

The foregoing may be summarized by saying that there can be no doubt that all the patients in this study had on admission to the Kankakee State Hospital positive neurologic, mental, and laboratory findings of general paralysis, and hence all had at one time had syphilis. Further, all of the patients had been given some form of treatment, and in some, there resulted a reversal of the laboratory findings from positive to negative. In others the serologic find-



TABLE III  
KAHN VARIATIONS

DATE, 1934	VI J.M.	VII M.K.	VIII F.W.	IX F.K.	X G.T.	XI H.E.	XII L.M.
January 14	±	++++	+++	-	++++		
15							
16	+	++++	++++	-	++++		
17							
18	+	+++	-	++++	++++		
19							
20							
21	-	-	++	-	++++		
22							
23	-	++	++	-	++++		
24							
25	±	++	+++	-	++++		
26							
27							
28	++	++++	+++	-	++++		
29							
30	+	++++	+++	-	++++	++++	+++
31	++	++++	+++	-	++++	++++	+++
February 1	++	++++	+++				
2							
3							
4	-	+++	+	-	++++	+	+++
5							
6	-	++	+++	-	++++	++++	+++
7							
8	++	++++	++++	-	++++	++++	++++
9							
10							
11	±	++	+++	-	++++	++++	++++
12							
13	-	++++	++++	-	++++	++++	++++
14							
15						++++	++++
16							
17						++++	++
18							
19						++++	++
20							
21						++++	+++
22							
23							
24						++++	+++
25							
26						++++	++
27							
28						++++	++++
March 1							

ings remained positive, and in still others there was noted a marked variation. The interest lies in the group comprising those patients on whom were noted variations in the serologic reactions. Apparently from the results, it is quite possible for a patient to have a four-plus Kahn or Wassermann on one day, and a few days later to have negative reactions.

Since it was believed that the variations were due to changes in the serum, and not due to laboratory error, an explanation was sought for in the organism itself. Since syphilis is a chronic progressive disease process, it does not seem reasonable to believe that the activity of the process underwent such rapid and marked changes. Hence it was thought that there were other factors which

underwent rapid changes, and in that way influenced the serology. On the basis of past work dealing with the effect of meteorologic changes on the organism, one of us (W. F. P.) has noted a close relationship between these changes, and the activity and physiologic functions of man. In order to establish the presence or absence of a correlation between the serologic variations and meteorologic changes, the meteorologic state has been compared with the serologic variations as follows: Thus, from January 13 to 17 there occurred a polar infall with a rising barometer. This episode is labeled 1 and 2 over the barograph. In Patient X the Wassermann reaction became positive on the fourteenth and eighteenth, i.e., immediately after the maximum of each meteorologic event. Patient IX became positive (Wassermann one-plus, Kahn four-plus) on the eighteenth. Patient VIII became positive to both the Kahn and Wassermann on the fourteenth and sixteenth. Patient VII had a positive Kahn on the seventeenth, and Patient VI became doubtful with Wassermann on the sixteenth and eighteenth, doubtful Kahn on the fourteenth, and one-plus Kahn on the sixteenth and eighteenth.

From January 23 to 26 two polar infalls occurred in rapid succession (labeled 3 and 4 on the barograph). In Patient X a doubtful Wassermann was recorded on the twenty-fifth. In Patient IX there was no alteration. In Patient VIII the Wassermann was unaltered, but the Kahn was increased on the twenty-fifth. In Patient VII the Wassermann became doubtful on the twenty-fifth

TABLE IV

DATE, 1934	WASSERMANN			KAHN		
	XIII J.B.	XIV L.C.	XV J.W.	XIII J.B.	XIV L.C.	XV J.W.
January 30	++++	++++	++++	++++	++++	++++
31						
February 1	++++	++++	++++	++++	+++	++++
2						
3						
4	+++	++++	++++	++++	+++	++++
5						
6	++++	++++	++++	++++	++++	++++
7						
8	++++	++++	++++	++++	++++	++++
9						
10						
11	++++	++++	++++	++++	++++	++++
12						
13	++++	++++	++++	++++	++++	++++
14						
15	++++	++++	++++	++++	++++	++++
16						
17						
18	++++	++++	++++	++++	++++	++++
19						
20	++++	++++	++++	++++	++++	+++++
21						
22	++++	++++	++++	++++	+++	++++
23						
24						
25	++++	++++	++++	++++	+++	++++
26						
27	++++	++++	++++	++++	++++	++++
28						
March 1	++++	++++	++++	++++	++++	++++

and the Kahn became positive on the twenty-third and twenty-fifth, and in Patient VI the Kahn became doubtful on the twenty-fifth.

A major polar infall began on the twenty-eighth<sup>5</sup> and reached maximal extent on the twenty-ninth and thirtieth of January with zero weather. Patient

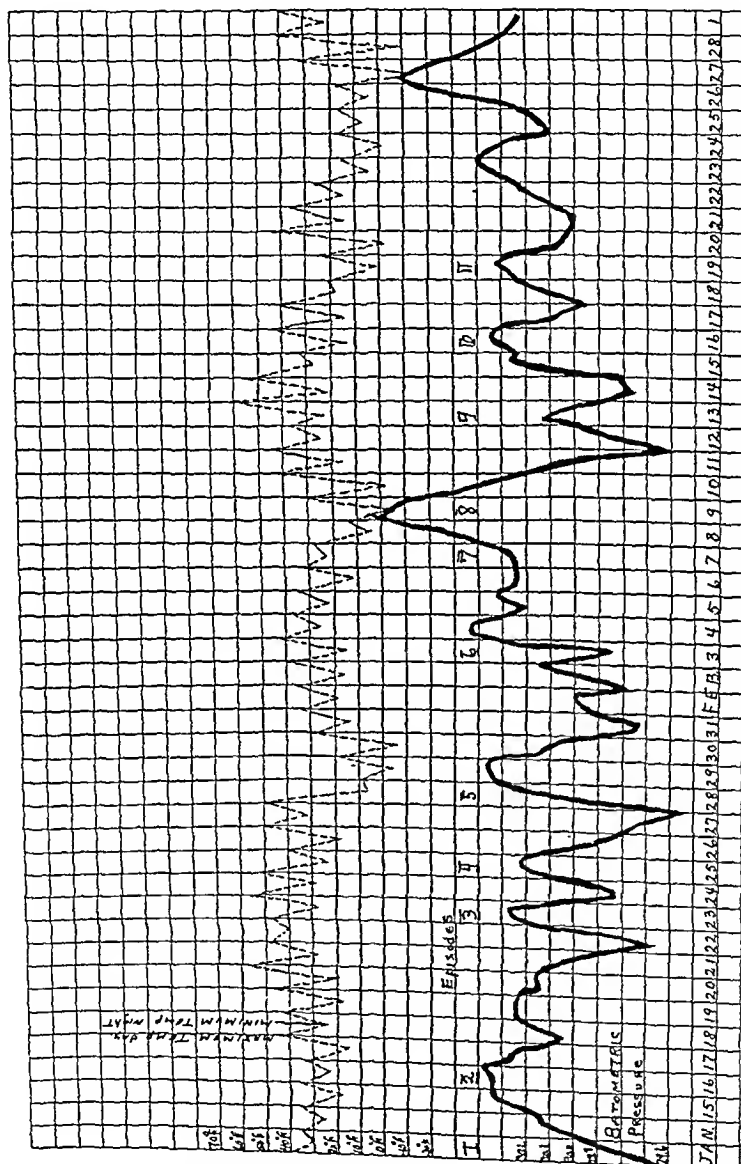


Chart 1.

XI had a two-plus Wassermann on the thirtieth, Patient X had a four-plus Wassermann on the thirtieth, and on February 1. Patient IX was unaltered, Patient VIII had a two-plus Wassermann on the thirtieth and a one-plus on the first. Patient VII had a four-plus Kahn on the twenty-eighth, and a two-plus on the thirtieth, and a four-plus on the first. He had a two-plus Wassermann on the thirtieth, and a one-plus on the first. Patient VI had a two-plus Was-

sermann and Kahn on the twenty-eighth, one-plus Wassermann on the thirtieth and first, and a one-plus Kahn on the twenty-eighth, and a two-plus on the first.

The next great disturbance followed in the form of three polar infalls that culminate in unusually high barometric pressure. These episodes have been labeled 6, 7, and 8. The reaction in Patient XI was unaltered, Patient X had a one-plus Wassermann and a doubtful Wassermann on the sixth and eighth. The reaction in Patient IX was unaltered, and Patient VIII developed a two-plus Wassermann and a four-plus Kahn. Patient VII had a three-plus Wassermann on the eighth, and a four-plus Kahn at the same time. Patient VI developed a doubtful Wassermann and a two-plus Kahn on the eighth. Probably as a delayed reaction to the stimulus we found the positive Wassermanns of Patients X, IX, and VI on the eleventh.

A series of less intense meteorologic changes now followed with polar infalls numbered 9, 10, and 11. In Patient XI the effects could be readily followed because the observations were continued and each polar infall was associated with a change in the serologic reaction.

It is not our purpose at this time to enter into a discussion of the mechanisms here involved, since the study is part of related observations in other field;<sup>2</sup> we wish merely to call attention to the fact that even such seemingly certain serologic reactions as those of syphilis are subject to the constant biologic tide as it is conditioned by the meteorologic environment. In later studies we shall present further details.

Chart I shows in detail the facts just discussed. The chart shows the changes in the barometric pressure, and external temperature during the period from January 13 to March 1, 1934, inclusive. The dotted line indicates the various temperature changes. This includes the maximum and minimum temperature readings. It so happens that the maximum readings occurred during the day, and the minimum occurred during the night. The heavy shaded line shows the changes in the barometric pressures. The figures on the left hand side indicate the readings in inches of mercury. Above the barograph, are number episodes which have been compared with the serologic variations.

#### CONCLUSIONS

1. A single blood Wassermann or Kahn test, if negative, means little or nothing as far as ruling out syphilis is concerned.
2. There occurs in some individuals having syphilis rather striking variations in the serology, and these variations take place in terms of days.
3. In those cases where variations occur, there seems to be no absolute agreement between the Wassermann and Kahn tests.
4. It is believed that the variations in the serology are conditioned by meteorologic changes.

#### REFERENCES

1. Hoverson, Emil T.: The Reversal of the Blood Wassermann Reaction in Untreated Syphilis, *Am. J. Syph.* 17: 392, 1933.
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## CREATININE CLEARANCE DURING THE HYPERTHERMIA OF DIATHERMY AND FEVERS\*

WILLIAM H. GRANT, M.D., AND GRACE MEDES, PH.D., MINNEAPOLIS, MINN.

ALTHOUGH Bieter (1933) observed that rate of blood flow to the glomerulus and rate of glomerular filtration in a frog's kidney perfused by Richard's method, were increased by heating the afferent artery, the effects of general systemic hyperthermia have been a subject of some disagreement. McIntosh and Reimann (1926) found that out of 13 cases of pneumonia, 9 patients showed renal hyperfunction as demonstrated by the phenolsulphonaphthalein test and the urea concentration index, while 4 showed a moderate depression. In a review of the literature dealing with renal function in pneumonia, these authors found that out of 6 investigators, 3 reported impairment of kidney function, 2 reported absence of function and one emphasized the occurrence of renal hyperfunction. Goldring (1931) in acute rheumatic fever, found urea clearances more elevated than the highest normal values. Ambard (1920) lowered the temperatures of dogs by wrapping their shaved abdomens in ice and found the uremic debit and the ureo-secretory constant lowered.

In view of the discrepancies of these findings, we have studied (A) creatinine clearances in dogs during the hyperthermia produced by diathermy and (B) creatinine clearances in patients with fever caused by infection to determine (A) the effect of general systemic hyperthermia on creatinine clearance and (B) the extent to which any changes observed in infectious fevers may be referred to the elevation of body temperature.

### EXPERIMENTS

*A. Hyperthermia of Diathermy.*—For the diathermy experiments male dogs varying from 13 to 21 kg. body weight were employed. No anesthetic was used, the dogs having been previously trained to lie quietly throughout the procedure, strapped to a dog board in a comfortable position. The technic of the test was the same as that described by Rehberg (1931) for clinical use, except that 2 gm. of creatinine dissolved in 10 to 15 c.c. of warm saline solution were injected into the saphenous vein before starting. The dogs were then given 250 c.c. of warm water by stomach tube to ensure adequate diuresis. All urine was taken by catheter and the bladder washed repeatedly with warm saline solution until the washings were clear.

The type of diathermy apparatus employed was described by Hemingway and Stenstrom (1932). The electrodes which consisted of two copper-mesh plates attached to thick pieces of cotton padding, were soaked in 1 per cent saline

\*From the Department of Medicine, University Hospital.  
Received for publication, May 3, 1934.

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solution and applied to the shaved sides of the thorax and abdomen of the dogs. These pad-electrodes were kept moist with saline solution throughout the experimental period.

At the beginning of the test the dog's rectal temperature was taken. The electrodes were then connected to the diathermy machine and the current turned on. Medium voltage and a current ranging between 1,000 and 2,000 ma. were used. The dog was covered with a blanket to prevent excessive heat loss and when the temperature was elevated from  $0.8^{\circ}$  to  $1.5^{\circ}$  C., a process requiring upward of ninety minutes, the test was begun. Four dogs were employed and 6 to 8 experiments made on each dog, 3 or 4 at normal temperature (control periods) and a similar series using diathermy. Rest periods of several days were allowed between tests.

*B. Hyperthermia in Fevers of Infectious Origin.*—Patients with hyperthermia (any temperature above  $37.2^{\circ}$  C.) were tested by Rehberg's method, both during the febrile period and after their temperatures had become normal.

## RESULTS

*Filtration and Temperature.*—The results of the diathermy experiments are given in Table I. There was in each case an increase in the creatinine clearance during diathermy, but no correlation between the average number of degrees the temperature of the different dogs was raised and the increase in their

TABLE I

RESULTS OF DIATHERMY EXPERIMENTS ON 4 DOGS, GIVING GLOMERULAR FILTRATIONS (CREATININE CLEARANCES) WITH RECTAL TEMPERATURES (1) BEFORE AND (2) DURING THE TESTS. EACH FIGURE IN THE TABLE REPRESENTS THE AVERAGE OF THE FINDINGS OF 3 OR 4 SEPARATE EXPERIMENTS WITH INTERVALS OF ABOUT 3 DAYS BETWEEN TESTS

DOG	BODY WEIGHT KG.	NO. TESTS	DIATHERMY	TEMPERATURE		FILTRATION C.C./MIN.
				1 ° C.	2 ° C.	
1	16.0	3	Without	39.1	39.1	78.7
		3	With	39.2	39.3	94.5
2	13.2	3	Without	38.3	38.3	36.6
		4	With	38.6	39.6	78.6
3	20.9	4	Without	39.2	39.2	79.2
		4	With	39.1	40.1	94.0
4	15.5	4	Without	38.6	38.7	60.0
		4	With	38.9	40.0	75.5

creatinine clearances. Thus, Dog 1, with an average elevation of temperature of  $0.6^{\circ}$  C. had an average increase in filtration of 15.8 e.c. per minute, while Dog 2, with an average rise in temperature of  $1^{\circ}$  C. had an increase in filtration averaging 42 e.c. per minute. There was, however, a correlation between the two factors in each individual dog. For instance, three tests with diathermy were made on Dog 1, with increase in temperature of  $0.3^{\circ}$ ,  $0.7^{\circ}$  and  $1.1^{\circ}$  C., respectively, while the corresponding filtrations increased 8.8, 13.1, and 25.6 e.c. per minute. These findings are similar to those of Medes and Herrick (1933) for the relation between blood flow to the kidney and creatinine clearance, where a general parallelism was found to exist between the two phenomena in each individual dog, although the same close relationship did not hold between the clearances and blood flow in different dogs.



The results of the experiments with fever due to infection are given in Table II. There was no indication of a general increase or decrease in kidney function when the temperature was moderately elevated. However, in every case studied in which the temperature was raised to 38.9° C. or above there was increased filtration. In those cases (Cases 4, 6, 7, 11, 13) in which there was the greatest increase in kidney function during hyperthermia, the elevation in temperature ranged from 1.0° to 2.3°. In the three cases of pneumonia (Cases 1, 14, 15) the filtration was increased during the period of fever.

TABLE II

TEMPERATURES, BLOOD PRESSURES AND GLOMERULAR FILTRATIONS (CREATININE CLEARANCES) OF 15 PATIENTS (1) DURING AND (2) FOLLOWING FEVERS OF INFECTIOUS ORIGIN

PATIENT	CLINICAL DIAGNOSIS	AGE YR.	TIME BET. TESTS DAYS	TEMPERATURE ° C.		BLOOD PRESSURE MM. HG		FILTRATION C.C./MIN.	
				1	2	1	2	1	2
1	Bronchopneumonia	47	6	38.3	36.9	100/68	96/66	146.9	90.4
2	Agranulocytic angina	56	13	39.0	36.7	116/64	96/60	262.2	73.1
3	Pyelonephritis, cystitis	22	10	38.9	36.6	140/100	108/76	187.4	124.5
4	Neurosyphilis, malarial fever	56	12	38.6	36.9	110/65	104/60	186.5	142.3
5	Cardiorenal nephritis	45	6	37.8	36.9	170/140	162/128	63.3	77.4
6	Sexual neurasthenia	22	6	38.2	37.0	136/84	124/80	176.6	102.1
7	Hypertension, cardiac decompensation	56	6	38.1	37.1	164/84	154/90	132.8	19.6
8	Pleurisy with effusion	49	6	38.7	37.0	134/86	120/74	67.9	110.2
9	Polyarthrititis	20	10	38.3	36.6	100/80	115/80	120.8	166.4
10	Diphtheria	27	7	38.1	36.3	124/80	110/80	155.8	173.6
11	Hodgkin's disease	60	22	38.3	36.9	104/60	96/58	236.6	107.5
12	Chronic nephritis, dermatitis	29	7	37.9	37.0	126/64	124/64	69.0	77.4
13	Chronic cholecystitis	31	8	37.9	36.7	130/80	102/68	144.2	92.5
14	Lobar pneumonia type III	24	13	40.5	36.8	130/72	120/70	545.2	174.0
15	Hypertension	32	15	38.9	36.9	132/96	126/92	222.6	132.1

As in the diathermy experiments, the differences in the filtrations during hyperthermia and after the temperatures had subsided to normal, bore no definite relation to the differences in temperature. For instance, Case 4, with a difference in the temperature of 1.7°, showed only a decrease of 44 c.c. per minute in the filtration, while Case 7, with a difference of but 1°, decreased 113 c.c. per minute in filtration.

*Filtration and Body Weight.*—It has previously been shown by Medes and Berglund that there is no correlation between body weight and rate of glomerular filtration in adult human beings under the conditions of Rehberg's test. That such a general relationship holds for large groups, however, may be seen by comparing the filtration per kilo body weight of man and dogs at approximately the same body temperature. For this purpose the filtration rates of dogs at normal temperatures (range from 38° to 39.4°, average 38.8°) may be compared with the rates of patients with infectious fevers (range from 37.8° to 40.5°, average 38.7°). The average filtration rate per kilo of body weight for the dogs was 3.9 c.c. per minute and for the patients 3.1 c.c. per minute. The same patients at normal temperature (36.8°) had an average filtration rate of 1.8 c.c. per minute per kilo body weight, and the dogs during diathermy (average temperature, 39.9°) had filtration rates of 5.2 c.c. per minute. Medes and

Berglund, in the study mentioned above, found an average filtration rate per kilo body weight of 2.4 in sixty normal medical students. Had a group with this filtration rate been subjected to a rise of temperature similar to that of the patients, it seems probable that a value more nearly approaching the 3.9 found on dogs would have been obtained.

The increase in the rate of filtration for each degree rise in body temperature varied considerably and showed no constant relation to body weight. Thus, Dog 2, which weighed 13.2 kg., had an average increase of filtration per degree rise in temperature of 42 c.e. per minute, whereas the heaviest dog (weighing 20.9 kg.) had a rise in its creatinine clearance of only 14.8 c.e. per minute per degree elevation of body temperature, and for Dog 4, weighing 15.5 kg., the corresponding increase in filtration was 14.1 c.e. per minute. The average increase in filtration for each degree rise in temperature found in sixteen experiments on the dogs was 10 c.e. per minute, a rise of 28 per cent. The corresponding average increase in the patients was 25.7 c.e. per minute, an increase of 23 per cent.

The striking agreement in filtration rates per unit body weight at approximately the same temperature in dogs and man, together with the agreement in the percentage increase in filtration per degree rise in temperature, indicates that in measurements of filtration we are dealing with a process fundamentally related to body metabolism.

*Filtration and Blood Pressure.*—Medes and Bellis (1933) showed a direct relationship between blood pressure in the renal artery and glomerular filtration in dogs, as measured by the creatinine clearance test. Nine of our cases of fever due to infection in which there was some increased filtration during fever also showed some increase in the blood pressure during this period (Cases 1, 2, 3, 6, 7, 11, 13, 14, 15). Patient 5 who showed renal impairment during both tests, had a slightly lower clearance during hyperthermia than after, while the blood pressure was slightly higher during hyperthermia. Case 9 showed both a decreased filtration and blood pressure during hyperthermia. Since this case was in a single class a short history is given: female, aged twenty, entered the hospital two weeks postpartum suffering with an acute polyarthritis. The usual kidney function tests were within normal limits. The Rehberg test was also normal, showing a filtration of 120.9 c.e. per minute. The temperature at the time of the test was 38.3° while the blood pressure was 100/80. Ten days later when the temperature had dropped to 36.6° the test was repeated. At this time the filtration was found to be 166.4 c.e. per minute, while the blood pressure was 115/80. The findings in this case conform to the work of Janney and Walker (1932) who concluded from a water diuresis test that there is generally a decreased kidney function at the time of delivery, and that this kidney function gradually increases postpartum until it again reaches its normal value.

Ellis and Weiss (1933) studied 18 cases of arterial hypertension by the Rehberg test. Out of this series 13 showed normal results, two showed slight reduction in function, while three showed marked reduction. No mention of temperature was given in their report. Two of the cases studied in our series had hypertension (Cases 5 and 7). Case 5 remained below normal during both

tests, while Case 7 dropped from a clearance well within normal limits during hyperthermia to one markedly below normal after the hyperthermia had disappeared.

### CONCLUSIONS

In contrast to the general parallelism that exists between body temperature and creatinine clearance in dogs during diathermy when all other factors are being held as nearly constant as possible, the creatinine clearances in patients with fever undergo wide variations. In the series of 15 patients observed here, the clearances during the height of the fever varied from 600 to 60 per cent of their postfebrile values.

A survey of the relationship between blood pressure and filtration during the fever, revealed similar irregularities: nine patients showed elevation of all three factors, temperature, blood pressure, and filtration; three showed increase in blood pressure with fall in filtration; in one patient filtration and blood pressure were lowered, while in the remaining two cases the changes in blood pressure and filtration were insignificant.

The group whose behavior is the most difficult to explain on a theoretical basis is that in which blood pressure and temperature were both elevated while filtration was lowered. Two other known factors may be considered as responsible for this behavior: (a) there may be a decrease in the volume flow of blood to the kidney in spite of elevation of temperature and blood pressure, and (b) there may be a direct effect of the infection on the kidney, resulting in a temporary closing off of some of the glomeruli or in an altering of their permeability.

The authors wish to express their thanks to Dr. Allan Hemingway of the Department of Physiology for the use of his diathermy apparatus.

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## THE EFFECT OF ALCOHOL UPON SPLEEN VOLUME\*

F. D. MCCREA, PH.D., D. F. MARION, B.S., W. W. TYSON, AND  
W. P. KAVANAGH, A.B., DURHAM, N. C.

**M**OST investigators agree that small quantities of alcohol cause dilatation in the cutaneous area and constriction in the splanchnic area, the latter to a somewhat lesser degree. The blood pressure may either show no change or be slightly increased or diminished (Dixon,<sup>1</sup> Brooks,<sup>2</sup> Stewart,<sup>3</sup> Lieb,<sup>4</sup> Hyatt,<sup>5</sup> McDowall<sup>6</sup>).

Few references concerning the action of ethyl alcohol upon spleen volume appear in the literature. Barcroft and Rothschild<sup>7</sup> reported that alcohol administered by stomach to unanesthetized dogs had little effect upon the spleen until large doses (8 c.c. of alcohol per kilogram) were given, when the spleen gradually decreased in size, the maximum diminution, amounting to 42 per cent, occurring some twenty minutes later. Definite signs of alcoholic intoxication were noted. In cases where no constriction was noted, the animals were apparently not intoxicated by the dose used. Karitsky<sup>8</sup> states that while alcohol paralyzes the central nervous system, it almost never abolishes splenic contractions.

The following report presents further data concerning the action of small quantities of ethyl alcohol upon the motility of the spleen.

*Method.*—Cats were used. They were anesthetized with urethane intraperitoneally and prepared to register blood pressure and spleen volume as in preceding work.<sup>9</sup> Alcohol 1 c.c. in concentrations varying from 2 to 20 per cent was administered via the femoral vein.

*Results.*—With threshold or slightly higher doses of alcohol, systemic blood pressure always fell slightly and quickly recovered, while spleen volume invariably diminished, its degree of change varying more or less directly with the concentration of the alcohol. Rhythmical movements of the spleen also occurred after the initial contraction, resembling those obtained with other drugs (Schaffer and Moore<sup>10</sup> and Barcroft and Nisimarn<sup>11</sup>). These results were noted in a total of 34 experiments with 8 cats. There seemed to be less relationship between the concentration of alcohol and the blood pressure, since in only a few cases was there an appreciably greater drop with 20 per cent than with 5 per cent alcohol. With larger doses (2 to 4 c.c. of 15 or 20 per cent) there was in most cases a greater fall and less prompt recovery.

In Fig. 1, *A* is seen the result of a dose of 1 c.c. of 5 per cent alcohol. Fig. 1, *B* shows the action of 1 c.c. of 20 per cent alcohol. The vascular depression is approximately equal in each and recovery almost equally prompt. How-

\*From the Department of Physiology and Pharmacology, Duke University Medical School. Received for publication, May 19, 1934.

ever, the degree of contraction of the spleen is much increased by the higher concentration of alcohol. Furthermore the rhythmical contraction and relaxation is much more pronounced and the spleen has a tendency to retain a higher tonus than that obtaining before alcohol, or than with concentrations less than 10 per cent, in which return to the control volume was almost always complete.

*Discussion.*—Henning<sup>12</sup> reported that abolition of the influence of the central nervous system by urethane narcosis stops the contraction of the spleen in mice and rats. Urethane has been the anesthetic routinely used in investigating the motility of the spleen in cats over a period of several years. At no time has urethane, when administered in sufficient quantity to secure surgical anesthesia, altered the splenic response to any very evident extent. Deep anesthesia undoubtedly does reduce the celerity and magnitude of its response, a point noted by De Boer and Carroll<sup>13</sup> and when combined with ether causes also some reduction of systemic blood pressure, as Barcroft<sup>14</sup> has pointed out. The ideal

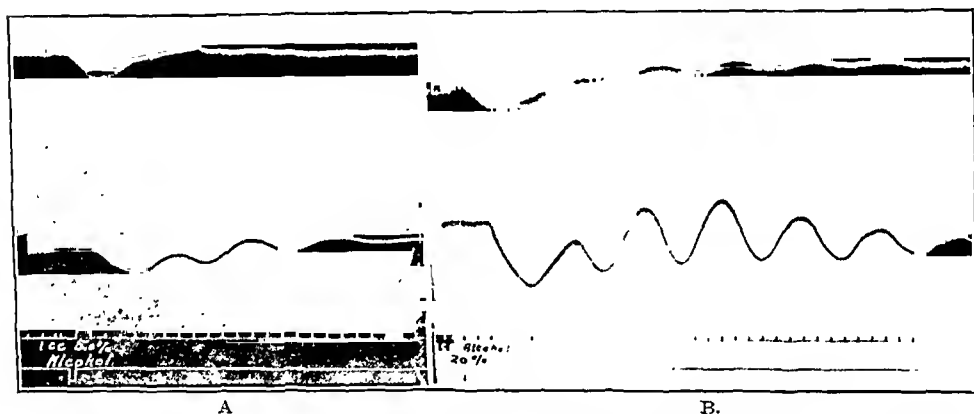


Fig. 1.—A, Effect of 1 c.c. of 5 per cent ethyl alcohol. B, Effect of 1 c.c. of 20 per cent ethyl alcohol. Tracings from top to bottom, carotid pressure, spleen volume, time six second intervals and signal markers denoting injection.

method of ascertaining the effects of drugs undoubtedly necessitates an animal which is not anesthetized. However, so far as the splenic reaction to various drugs has been studied on anesthetized and unanesthetized animals the response is in the same direction according to most investigators. Contrary to Barcroft and Rothschild<sup>7</sup> we find that small intravenous doses of alcohol produce considerable contraction of the spleen, although with large doses our results are substantially the same as theirs. A possible explanation of this difference lies in the mode of administration, the effective stimulus probably being the concentration of alcohol in the blood. Brooks<sup>2</sup> demonstrated that alcohol introduced into the stomach by way of a gastric fistula caused no immediate vascular response, but a gradual reduction of systemic pressure occurred after some fifteen minutes. Likewise he found that slow intravenous injections of alcohol ranging from 0.5 to 4.0 c.c. per kilogram of animal were without effect other than cardiac acceleration and a gradual reduction of systemic pressure which invariably followed.

The rhythmical contractions produced in the spleen by alcohol, which are so pronounced in Fig. 1, *B*, occur in every record in which threshold or higher doses of alcohol are administered. These rhythmical waves are moreover seen to cause rhythmical fluctuations of the systemic blood pressure. The magnitude and duration of the splenic waves increased as the dose of alcohol used, occasionally not disappearing completely from the record for more than an hour after its administration.

*Summary.*—Small doses of dilute alcohol administered intravenously to cats anesthetized with urethane cause contraction of the spleen. This contraction is followed by rhythmical relaxation and contraction which may last for a considerable period of time.

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## SOME STUDIES ON THE ETIOLOGY OF GRANULOMA INGUINALE\*

HILDRUS A. POINDEXTER, M.D., WASHINGTON, D. C.

IN THE United States, with the exception of certain localized areas in some of the southern states, granuloma inguinale in the past has been referred to as a rare malady. Within more recent years, interest has been focused on this clinical condition primarily for three important reasons: First, the malady is becoming more prevalent and more generalized geographically; second, it is being considered by some as a fourth venereal disease; and third, there is a lack of agreement as to the nature of its etiology.

We have made microscopic examinations of nine clinical cases in Freedmen's Hospital within the last three years. Three of these patients lived within the vicinity of the District of Columbia for at least five years prior to the appearance of any lesion, and the other five came from various parts of the southern states.

Upon repeated smear examinations, seven of these nine cases showed the microscopic diagnostic picture of granuloma inguinale, when stained by the Wright or Giemsa method. The exact nature of these included bodies is not known.

Some observers agree with Donovan,<sup>1</sup> that it is a protozoan of the gregarine order of sporozoa; some with Flu,<sup>2</sup> that it is a chlamydozoan and there are others among whom are many of the more recent workers such as Campbell,<sup>6</sup> Randall, Small and Belk<sup>7</sup> who agree with Walker,<sup>3</sup> that it is a member of the Friedländer group of bacilli.

Five of these nine cases have been reported by Poindexter.<sup>4</sup> The other four cases were observed since. Three are still being treated at the Freedmen's Hospital, while one has been discharged as cured following tartar emetic and fuadin medication.

Within the last two years, attempts have been made to isolate and culture the organism in all cases that were positive by microscopic examination. Of the last four cases, three were positive microscopically. From the three positive cases of this set we were able to isolate identical organisms in two. By returning to the study of the first five cases, we were able to isolate a similar organism from one even though the patient had received considerable treatment and was improving nicely.

From the study of these three similar organisms, it is hoped that additional evidence may be shown as to the nature of the intracellular bodies (etiological agent) in granuloma inguinale.

\*From the Department of Bacteriology, Preventive Medicine and Public Health, College of Medicine, Howard University, and Freedmen's Hospital.  
Received for publication, May 17, 1934.

The organisms which form the basis of this report were obtained from three clinical cases of the disease: Cultures from the first of these patients, M. N., one of those reported by Poindexter,<sup>4</sup> show the following organisms on isolation and study: (a) A gram-negative diplobacillus; (b) a small gram-positive staphylococcus and micrococcus; (c) a short chain nonhemolytic streptococcus; (d) a diphtheroid; (4) a few large thick rods which proved to be Döderlein's bacilli; and (f) the original smear from the patient also showed a spirochete which along with a fusiform bacillus gave a picture resembling that of a Vincent's angina smear. This fusiform bacillus is believed to be the same as the Döderlein's bacillus isolated by culture.

Cultures from the area of ulceration of the second patient, J. B., showed a pure culture of an organism similar in morphology and culture to the organism (a) isolated from the first patient, M. N. Cultures from the third patient, R. F., who is now on the ward receiving treatment with fuadin, contained the following organisms: (a) A small gram-negative bacillus which resembled Friedländer's bacillus morphologically. (b) A small gram-negative coccus, which corresponded culturally to that of *Micrococcus ureae* of Cohen; the gram-negative staining character was the exception, and (c) an organism of the diphtherial group.

The organisms (a) of the first and third patient, and the pure culture from the second were selected as the organisms of special study. The fact that three similar organisms appeared in three clinical cases of granuloma inguinale and that in one of these cases a pure culture was found, is significant from an etiologic standpoint. The selection of these organisms for study is in accordance with the opinion of Walker,<sup>3</sup> 1918, DeMonbreun and Goodpasture,<sup>5</sup> 1933, and others. Their thermal death point was 51° for thirty minutes, and 59° for ten minutes. The organisms did not show capsule formation in culture. Their biochemical reactions are shown in Table I.

#### EXPERIMENTAL

Fourteen mice and nine guinea pigs were used for the pathogenicity test of the organism in an attempt to fulfill Koch's postulates. Five mice were inoculated intraperitoneally with a suspension of the diplobacilli isolated from the first case; three of them with a saline suspension of a twenty-four-hour agar slant culture and the other two with  $\frac{1}{4}$  c.c. of a twenty-four-hour broth culture. The mice showed no ill effects from the inoculation. After four days one of the mice was killed and cultures were made from the peritoneal cavity and the heart's blood. The peritoneal cavity showed considerable polymorphonuclear exudate and the culture was positive for the diplobacilli, but the culture from the heart's blood was negative.

Another one of the mice was sacrificed on the ninth day, with negative results. The other three mice continued to live without any ill effects for two months, after which they were used in a trypanosome experiment. Four mice were used in a similar way to test the pathogenicity of the diplobacilli isolated from the second case. The pathogenicity results were negative. Similarly five mice were used for intraperitoneal and sacrifice tests with cultures from the



third patient with similar negative results. The guinea pigs were inoculated with doses twice the size of those given the mice. Three guinea pigs were used for each organism. The guinea pigs showed no reaction for eight days, after which time they appeared to be ill. Aseptic puncture of the peritoneal cavity resulted in the removal of some exudate similar to the type observed in mice, but the cultures for the organisms were negative. Similar punctures of the heart gave negative cultures. Within the following three weeks, the animals showed progressive loss of weight which resulted in marked emaciation and death about four weeks from the date of inoculation. Cultures taken by punctures from the peritoneum and heart were negative eight days after injection and remained negative as the emaciation progressed. By repeated experiments we were able, however, to obtain positive cultures of the organisms from fluid drawn from the peritoneum within the first six days after inoculation. The stools and urine were negative for the organism. At the autopsy there were no ulcerations or areas of granulation which in any way resembled those seen in granuloma

TABLE I

THE BIOCHEMICAL REACTIONS OF THE GRAM-NEGATIVE DIPLOBACILLI OBTAINED FROM THREE CASES OF GRANULOMA INGUINALE

MEDIA	FIRST PATIENT	SECOND PATIENT	THIRD PATIENT	OTHER CHANGES IN THE CULTURES
Dextrose	a*	a	a	Turbidity and a gray yellow fine granular precipitate.
Dextrine	-	-	-	A slight surface film and precipitate.
Maltose	a	a	a	A heavy yellow precipitate and a surface film.
Saccharose	a	a	a	A gray yellow precipitate and a surface film.
Lactose	a	a	a	A stringy granular flocculation.
Salicin	a	a	±	A thick gray film on the surface.
Mannite	a	a	a	Slight surface film and a gray yellow precipitate.
Inulin	-	-	-	Granular flocculation with a slight gray precipitate.
Inosite	-	-	-	A slight surface film and gray precipitate.
Sorbite	-	-	-	A slight surface film and slight precipitate.
Xylose	-	-	-	
Litmus milk	Sl.a*	Sl.a	a	No coagulation.
Plain agar	Grayish yellow slightly elevated. Glistening round colonies.			Similar reactions were observed for each of the other two organisms.
Peptone water	No indol formation.			Similar reactions were observed for each of the other two organisms.
Nutrient broth	Cloudy growth in 24 hours.			Similar reactions were observed for each of the other two organisms.
Gelatin	Not liquidified but bubbles of gas occur just below surface.			Similar reactions were observed for each of the other two organisms.
Nitrate reduction	±	±	+	
H <sub>2</sub> S test	-	-	-	
Methyl red	±	Sl.+	+	
Voges-proskauer	-	-	±	

\*a. Fermentation.

Sl.a. Slight acid without gas.

inguinale. The lung and gastrointestinal tracts grossly showed no lesions that could be considered the cause of death. Sections were not taken. Because this organism had resembled the Friedländer's bacillus in many respects, we performed comparative agglutination, absorption and precipitation tests between this organism and strains of Type II pneumococci. The results show that this organism is not similar to the Type A, B, or C of Friedländer's bacillus. However, we could not exclude it from the heterogeneous Group X of Friedländer's bacilli.

Guinea pigs and mice were also injected intraperitoneally with mixed cultures of these isolated organisms and gonococci. The results were similar to those obtained in the animals infected with the isolated organism alone.

#### DISCUSSION

We have observed in the nine cases which we recently studied here, and a review of many of the reported cases, that a concomitant gonorrheal and an occasional chancreoid infection were frequently present or were revealed in the history as existing prior to the beginning of the symptoms of granuloma inguinale. It is also noted that frequently an operation for inguinal adenitis (buboes) was performed, which did not heal.

The two cases reported by Gruhitz<sup>9</sup> both gave a history of inguinal swelling which failed to heal; one after lancing and the other after pricking. A similar history is given by Shattuck, Little and Coughlin<sup>10</sup> in a report of three cases from Boston. The extension of this unhealed incision is common in the history of granuloma inguinale cases.

The tissue transplantation en masse from one person with the disease to another susceptible person as done by McIntosh,<sup>5</sup> does not permit one to draw any conclusion as to the specificity of the organisms involved, since several organisms may be present.

A survey of the reported cases shows a higher incidence among colored than white of granuloma inguinale in the United States. This is a statement of fact, the explanation of which is involved in a large number of contributory factors. Of the contributory factors, the most important in this malady as in syphilis is the greater lack of information in the nature of personal hygiene among the colored.

#### SUMMARY AND CONCLUSION

We have isolated identical organisms from three cases of clinical granuloma inguinale. These organisms resemble organisms of the Friedländer's group and appear similar to the inclusion bodies in cells of curettements taken from ulcerations of clinical cases of granuloma inguinale.

We believe that the "Donovan bodies" are bacteria. The organism is non-pathogenic for mice, which is a differential point from Type A or B of the Friedländer's bacillus.

I wish to express my appreciation to Mr. Malaku E. Bayen and Miss Ferris Warren, juniors in the Medical and Dental Schools, for their assistance in most of these biochemical tests.

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## STEREOSCOPIC ROENTGENOGRAPHY OF BEDRIDDEN PNEUMONIA PATIENTS\*

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PAUL C. HODGES, PH.D., M.D., AND THOMAS H. LIPSCOMB, M.D., CHICAGO, ILL.

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INVESTIGATORS in the field of pneumonia and physicians responsible for the care of patients suffering from this disease are generally aware of the great diagnostic value of roentgenograms. They need the help of the roentgenologist at the beginning of the disease, throughout its course and during convalescence, and in our experience are glad to accept him as an equal partner in the diagnostic problem. However, even the briefest experience in such work will convince any physician, whether he be roentgenologist or clinician, that the problem is difficult and the results not all that might be desired.

Early in the disease and again during convalescence the patient is able to come to the x-ray laboratory, sit up in front of a cassette changer and hold his breath during the making of standard quality stereoscopic films. This is impossible or at least highly undesirable during the active phase. Instead it has become common practice to bring a portable unit to the bedside or move the patient and bed to the x-ray laboratory and make single nonstereoscopic films with the patient recumbent. Such films are sometimes of astonishingly good quality but the lack of three-dimension vision is a serious handicap and the insertion of a cassette beneath the patient involves considerable discomfort for him.

Clinicians not intimately associated with the problem are apt to underestimate the degree of this discomfort, holding the erroneous belief that an x-ray examination of the chest is an innocuous procedure if the patient does not leave his bed. The patient knows better. He must be lifted into a partial sit-

\*From the Division of Roentgenology, Department of Medicine, University of Chicago.  
Received for publication, May 22, 1934.

ting position while a cassette is inserted behind his back, and when he is lowered into the recumbent position he finds this an uncomfortable resting place even in that rare instance in which correct position is obtained at the first trial and rearrangement of patient and film is unnecessary.

In the course of many years of association with O. H. Robertson, whose investigations lie largely in the field of clinical and experimental pneumonia, one of us has made repeated attempts to improve the technical conditions of chest raying during the active stage of pneumonia.

Most of our earlier devices were found to involve too much effort on the part of the patient but the apparatus here described allows us to make stereoscopic chest films of recumbent subjects without submitting them to appreciable exertion or discomfort. In spite of the fact that the rays pass through mattress



Fig. 1.—Roentgenograms of normal subject. H. H., aged thirty-three, x-ray No. 2297. A. (Serial 15) Regular technic, subject standing facing commercial cassette changer, 70 KV, 100 Ma., 72" distance, exposure time 6/20 second.

B. (Serial 13) Subject on back in pneumonia bed, radiation from beneath through mattress, 70 KV, 150 Ma., 72" distance, exposure time 8/20 second.

and bed clothing, films made under these conditions compare very favorably with those made in the conventional fashion, that is, with the subject standing facing a commercial cassette changer (Fig. 1).

As soon as a patient is suspected of having pneumonia he is transferred to a special pneumonia bed which he continues to occupy until there is no further need of special chest raying. This is a hospital bed\* in which a square section of the upper half of the bed spring has been cut away and replaced by a square of stretched canvas (Fig. 2). An ordinary cotton-felt mattress rests on the

\*These beds, originally in general use throughout our hospital, have recently been replaced by a newer type equipped with inner spring mattress and two crank-driven worm devices for raising and lowering head and foot. We have not yet succeeded in adapting these new beds for x-ray use because of the interfering shadows of coil springs and the cross-bars of the worm mechanism. We do not consider such adaptation impossible, particularly if it is undertaken by the manufacturer of the bed.

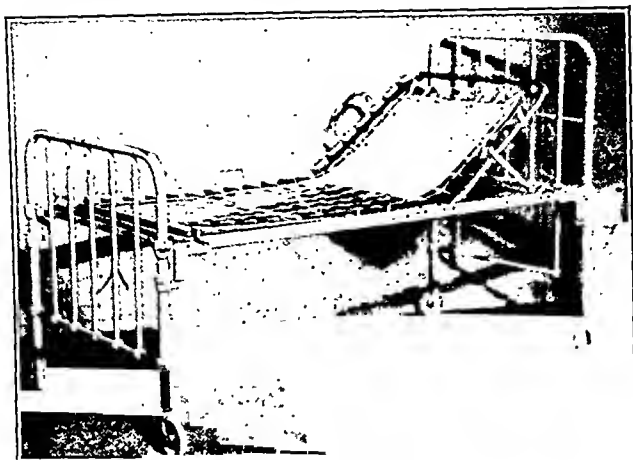


Fig. 2.—Pneumonia bed. A conventional hospital bed in which a section of spring has been cut away and the space filled in with a laced canvas window. In more modern type beds with worm mechanism for raising and lowering the head, rearrangement of the mechanism is necessary in order to provide a clear space beneath the chest. A regular cotton-felt mattress is used, care being taken that it contains no bits of wire or other metal objects.



Fig. 3.—X-ray tube suspended from ceiling in lower room. The cocking device for the stereoscopic shift, machine controls, etc., are in the room above. Protective wire caging has been removed to allow photographing the apparatus.

spring and except for the fact that there must be no rubber sheet beneath the patient's chest, the bedding is arranged and changed by the nurses in accordance with ordinary hospital practice.

Two x-ray rooms are required, one directly above the other. The lower room contains a mechanical rectifier x-ray machine mounted on a wall shelf together with a water-cooled 10 KW x-ray tube slung from a special stereoscopic tubestand attached to the ceiling (Fig. 3). The upper room, capable of darkening for fluoroscopy, contains the control stand and meters of the x-ray machine

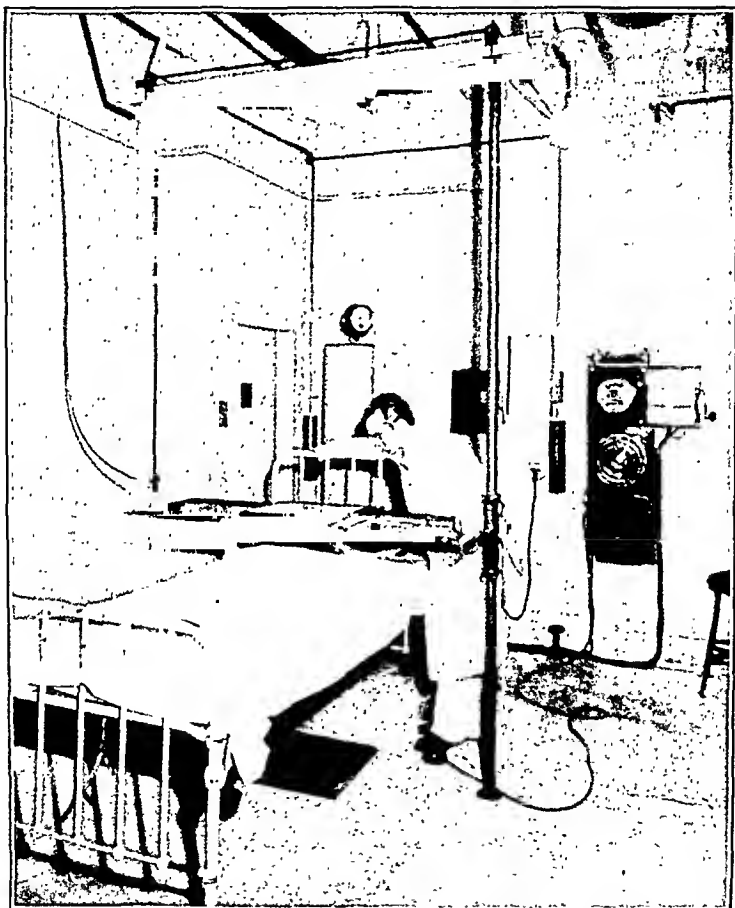


Fig. 4.—Patient on pneumonia bed in position for stereoscopic filming. The fluoroscopic screen is intended primarily for centering but with the addition of a shutter may be used for fluoroscopic observation of diaphragmatic excursion, etc.

and a special automatic cassette changer counterbalanced for easy vertical movement on two vertical rails. A rectangular, bakelite-covered opening through the floor transmits radiation from the tube lying directly beneath it, the tube target, center of floor port and center of film lying on a straight line perpendicular to the floor (Fig. 4). The patient recumbent on the pneumonia bed is wheeled into position above the floor port in the upper room, his head is turned to one side and the lowered cassette changer is brought down until the bakelite front of its central section touches the chest. The room is now darkened and

under fluoroscopic control, using radiation coming through the floor and a screen mounted on the upper surface of the central section of the cassette changer, the bed is moved about until the patient's chest is properly centered beneath the space presently to be occupied in succession by the two films.

During this fluoroscopic centering, which takes but a few seconds, the films are protected by the lead floor of the two outer sections of the changer. One cassette carriage is cocked against spring pressure and held by a stout trigger. The other, except for the light pressure of a friction catch, is free to roll along its track. This free carriage is now shoved into position between the bakelite window and fluorescent screen of the central section where it engages the cocked carriage and becomes attached to it, the machine controls are turned to the proper setting for filming and by means of a cable-and-lever arrangement the tube is cocked against spring pressure. It is now possible to make stereoscopic chest films employing speeds of exposure and film change about the same as those used with conventional vertical type cassette changers.

There are magnetic releases on tubestand and cassette changer and a motor-driven time switch times the exposures. Operation is further simplified by a five-pole, double-throw, externally operated switch. With the switch handle in the down position, the time-switch motor is off and the tube is set for long-distance fluoroscopy at 85 KV and 10 Ma. When the switch handle is up, the time-switch motor runs and the tube is ready to deliver 150 Ma. at 70 KV.

The apparatus meets a definite though limited need in our service. It seems to satisfy completely the requirements of stereoscopic chest filming at any phase of pneumonia unless pleural fluid be present and, in addition, renders valuable assistance in postoperative conditions where the clinical picture is complicated and it is important to know whether pneumonia or pulmonary infarct is present. To those who employ artificial pneumothorax in the treatment of pneumonia the apparatus offers a means for the frequent checking of the amount of pleural air and the degree of pulmonary collapse.

One of the leading x-ray manufacturers has constructed a neat and compact commercial model of our apparatus\* and commercial manufacturers of hospital beds could undoubtedly develop beds that would meet the x-ray requirements and at the same time be more comfortable than our homemade product.

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\*For the University of Michigan.

## THE RESPONSE TO INFECTION IN BONE MARROW DYSCRASIAS\*

FRANK H. BETHELL, M.D., ANN ARBOR, MICH.

CERTAIN changes in the constitutional environment of an individual, notably those caused by bacterial invasion, produce a more or less characteristic response on the part of the bone marrow. The neutrophile count provides a measure of the degree of such response. The functional capacity of the bone marrow to meet a sustained demand for more than usual numbers of neutrophiles is evidenced not only by the total number of neutrophiles per cubic millimeter but also by their stage of maturity. It is this conception which forms the basis of the Schilling<sup>1</sup> index and its interpretation. More recently the importance of immature white blood cell counts in infectious conditions has been emphasized by Reznikoff.<sup>2</sup> He points out that in many serious infections there is no increase in the circulating neutrophiles, but rather an apparent depression of the myelocytic response. The decrease in leucopoietic activity may be the specific effect of certain diseases, such as typhoid fever, tuberculosis, influenza, and malaria, or it may follow prolonged overstimulation in response to an overwhelming infection such as streptococcal septicemia. During recent years great interest has been shown in the phenomenon of extreme neutropenia which may develop in the presence of little or no demonstrable infection. If, as often happens in such cases, severe infection supervenes, the few neutrophiles found are predominantly immature in form. It is the relative percentage of immature and adult forms which indicates the effort of the bone marrow response as opposed to the total neutrophile count which measures solely the extent of the response.

The appearance in the blood stream of increasing numbers of young neutrophiles during the progress of an infection is dependent upon two factors, the state of the individual prior to the present illness, as reflected in the bone marrow, and the nature of the infection. Consequently, there are actually two variables, but in order to interpret the progress of an infection by studies of the neutrophile age the bone marrow must be thought of as a constant. In many instances, however, the neutrophile picture differs widely from what one would expect from the clinical course of the illness. The cases reported by Thompson,<sup>3</sup> in which there was either an overproduction of young and abnormal white blood cells or a marked decrease in the numbers of granulocytes in response to infection, fall into this group. The patients to be discussed in the present communication, likewise, showed an atypical bone marrow response in the presence of infection.

\*From the Thomas Henry Simpson Memorial Institute for Medical Research, University of Michigan.

Received for publication, May 11, 1934.

A summary of this paper was read at the New York meeting of the American Society of Experimental Pathology, on March 31, 1934.



While the diagnostic and prognostic value of the Schilling index and its modifications is limited to such cases as show a characteristic bone marrow response, the occurrence of cytoplasmic changes in the neutrophiles, and especially the appearance of granules which are basophilic to one of the Romanowski stains, is quite independent of the type of myelocytic reaction and will, in any case, give a reasonably accurate picture of the progress of an acute severe infection. Alterations in the staining characteristics of the cytoplasm of leucocytes have received considerable attention among German investigators,<sup>4, 5, 6, 7</sup> but elsewhere they have met with little systematic study except by Rosenthal and his coworkers.<sup>8, 9, 10, 11</sup> The reader is referred to these authors for morphologic descriptions of the phenomena under consideration. Basophilic granulation of the neutrophiles is not limited to infectious processes, but may occur in a wide variety of conditions in which there is marked alteration of the individual's internal environment with evidence of toxemia. Among such conditions may be mentioned reactions following transfusion with incompatible blood, intensive roentgen therapy, the toxemias of pregnancy, extensive malignancy with necrosis, and chronic myelogenous leucemia.

That these granules, in infectious states, are not merely a degenerative phenomenon, caused by exposure of the cells to a site of inflammation, is attested by the evidence gained from counts made on successive days during the exacerbation of an infection. When the total number of immature and mature forms exhibiting basophilic granulation are compared, it is shown that the granules are predominant in the cells most recently released into the circulation, and consequently, in all probability, the change occurs in the bone marrow. Table I illustrates a method by which the neutrophile counts may be tabulated

TABLE I

NEUTROPHILE COUNTS MADE ON THREE SUCCESSIVE DAYS SHOWING INCREASE IN PERCENTAGE AND ABSOLUTE NUMBER OF IMMATURE FORMS EXHIBITING CYTOPLASMIC BASOPHILIC ('TOXIC') GRANULATION. THE COUNTS WERE MADE ON CASE 1

DATE	MARCH 9		MARCH 10		MARCH 11	
	PER CENT	NO. PER C.M.M.	PER CENT	NO. PER C.M.M.	PER CENT	NO. PER C.M.M.
W.B.C. per c.mm.		4,800		7,100		9,450
Percentage of nonfilament neutrophiles	45		51		57	
No. per c.mm. nonfilament neutrophiles		2,160		3,620		5,390
Percentage of filament neutrophiles	45		41		41	
No. per c.mm. filament neutrophiles		2,160		2,910		3,870
Total percentage of neutrophiles	90		92		98	
Total no. per c.mm. of neutrophiles		4,320		6,530		9,260
Percentage of nonfilament forms with basophilic granulation	26*		39*		58*	
No. per c.mm. of nonfilament forms with basophilic granulation		1,120		2,530		5,400
Percentage of filament forms with basophilic granulation	28*		27*		14*	
No. per c.mm. filament forms with basophilic granulation		1,210		1,750		1,320
Total percentage of neutrophiles with basophilic granulation	54*		66*		72*	
Total no. per c.mm. neutrophiles with basophilic granulation		2,330		4,280		6,720

\*These percentages are of the total number of neutrophiles per cubic millimeter on the respective days.

in order to correlate the nuclear shift with the percentage and absolute number of basophilic granule cells. These counts were made on the patient reported as Case 1.

Blood examinations on the patients comprising the present study were made at frequent intervals throughout the period of hospitalization, and during the acute manifestations of their illness counts were taken daily. For purposes of simplification only the more significant findings will be reported. Leucocyte counts were done with pipettes and hemocytometers certified by the U. S. Bureau of Standards. Films were made on cover slips and stained with a fresh solution of Wright's stain, prepared in the usual manner without buffering. Control stains on blood from individuals without acute infection, or other evidence of toxemia, were carried out routinely. The polymorphonuclear neutrophils were classified according to their nuclear structure into nonfilament and filament groups as suggested by Cooke and Ponder.<sup>12</sup> However, the percentage recorded is of the total number of neutrophils rather than of the total leucocyte count. On this basis 6 per cent of nonfilament forms is an average normal value. On the charts only the nonfilament percentage is plotted, and this value, "the percentage of immaturity," is regarded as a measure of the effort of the bone marrow response. For graphic purposes basophilic granulation is recorded in terms of the percentage of neutrophils exhibiting this phenomenon, rather than in the form of a "degenerative index" as advocated by Kugel and Rosenthal.<sup>9</sup> The tabulation of at least 100 neutrophils was included in each differential. In the severe neutropenic states this usually required the examination of several films.

By the use in conjunction of the three lines of inquiry enumerated above, namely, the degree of bone marrow response as measured by the total neutrophil count, the effort of the response as indicated by the percentage of immature forms, and the severity of the infection as interpreted by the percentage of basophilic granule cells, it is hoped that some information may be gained concerning the physiology of the bone marrow in certain pathologic states. Incidentally, these studies confirm the prognostic value of the basophilic granule cell count.

#### CASE REPORTS

CASE 1.—M. N. Acute pansinusitis, cavernous sinus thrombosis and terminal streptococcal septicemia, without evidence of primary disease of the blood-forming organs. An American housewife of forty-eight years entered the hospital complaining of difficulty of breathing, cough, and weakness. For four years she had been subject to attacks of otitis media and acute sinusitis. Right mastoidectomy was done. The present illness commenced four months before admission with sore throat and right-sided frontal headache. Upper respiratory symptoms became increasingly severe, and during the two weeks prior to her entering the hospital small nodules developed in the skin of the trunk and extremities. On examination the patient appeared to be seriously ill. There was a mucopurulent discharge in the nose and throat and thickening of the mucosa of the nares and pharynx. The right membrana tympani was dull and reddened. Scattered over the trunk and extremities were numerous small cutaneous lesions with depressed bluish centers, surrounded by elevated yellow areas, which, in turn, were circled by zones of erythema. Temperature was 98° F.; pulse, 100; respirations, 27. The patient's condition did not improve, the temperature was continuously "septic" in type, localized suppuration occurred in the right periorbital tissues and

in the region of the right elbow. On the twentieth day of hospitalization she died. The red blood cell count on admission was 3,700,000 per c.mm.; hemoglobin, 59 per cent (Sahli); white blood cells, 7,300 per c.mm. Polymorphonuclear neutrophils were 78.5 per cent; eosinophiles, 2.5 per cent; large lymphocytes, 15.5 per cent; small lymphocytes, 2.5 per cent; monocytes, 1 per cent. On the day of death the white blood cell count fell to 1,750 with a neutrophilic percentage of 94. Leucocyte and neutrophil counts made on three successive days on this patient are given in Table I, and the character of the neutrophil response is plotted on Chart 1. The significant findings at necropsy in this case were streptococcus septicemia with focal necroses in skin, lungs, lymph nodes, spleen and liver, and acute parenchymatous degeneration of all organs. Microscopic examination of the marrow of the sternum and ribs revealed a congested mixed cellular and fatty marrow with numerous giant cells, and large areas nearly devoid of cells. The marrow of the ethmoid was almost completely exhausted of cellular elements with areas of necrosis and hemorrhage.

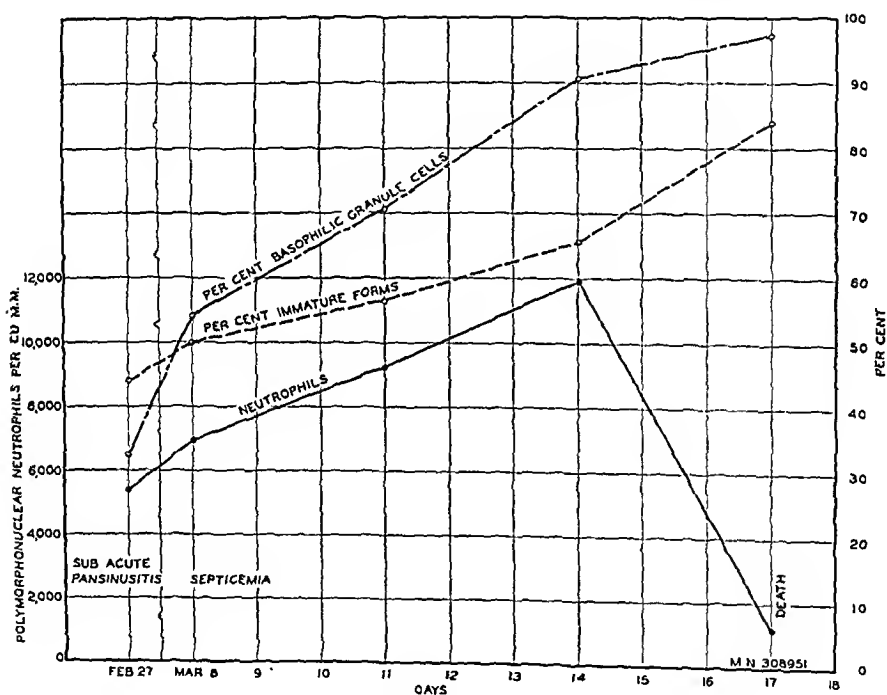


Chart 1.

This case is presented as an example of failing bone marrow response to an overwhelming infection. As no primary deterrent to leucopoiesis exists the effort of the marrow response, as measured by the percentage of nonfilament neutrophils, parallels the severity of the infection, plotted in terms of the percentage of neutrophils exhibiting basophilic granulation. The observations on this patient are to be contrasted with those made on cases whose infection complicated a primary bone marrow dyscrasia.

CASE 2.—S. M. Pernicious anemia in relapse, acute otitis media with mastoiditis, and acute cholecystitis and cholelithiasis. An American woman of fifty-two years, self-supporting, was admitted with a complaint of weakness, numbness and tingling of the extremities, and recurrent soreness of the tongue. The present illness was of eleven months' duration, the symptoms becoming gradually more severe. Four months before entering the hospital she began to suffer with attacks of pain in the right upper abdominal quadrant, accompanied by

nausea and vomiting, and at times followed by transient jaundice. Examination revealed pallor and icteric tint of the skin and mucous membranes, tenderness in the right hypochondrium, enlargement of the liver, and impairment of vibratory sense in the lower extremities. Temperature 100° F.; pulse 80; respirations 22. During the first three weeks of hospitalization tenderness and pain in the right hypochondrium persisted, there was an irregular fever of moderate degree, and no significant response on the part of the blood following treatment with ventriculin and intramuscular liver extract. Subsequently the patient complained of right earache; the drum was found to be reddened and bulging, and paracentesis was done with the release of much mucopurulent exudate. Tenderness over the right mastoid developed, and the temperature became markedly "septic" in character. The patient's condition was considered to be too poor for operation; blood transfusions were given with slight temporary improvement, but all efforts to overcome the infection were unavailing, and five weeks after admission she died. A necropsy was not obtained.

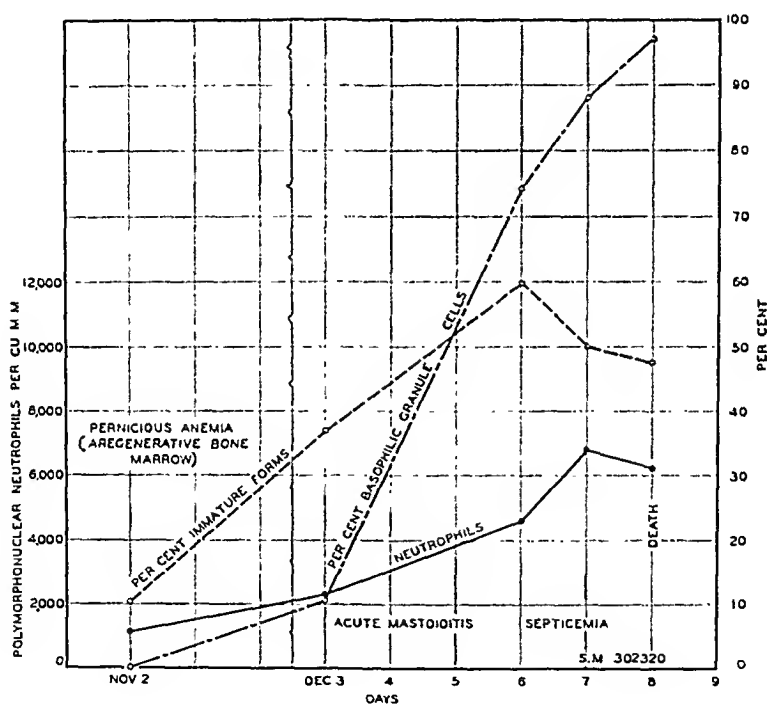


Chart 2.

The red blood cell count on admission was 1,130,000 per cmm.; hemoglobin, 29 per cent (Sahli); white blood cell count, 3,000 per cmm.; polymorphonuclear neutrophils were 38.5 per cent; large lymphocytes, 17 per cent; small lymphocytes, 43.5 per cent; monocytes, 1 per cent. The stained blood film revealed the morphologic characteristics of pernicious anemia with definite macrocytosis of the erythrocytes. Achlorhydria of the gastric contents was found after the subcutaneous injection of histamine hydrochloride 1.0 mg. Cholecystography following the intravenous administration of dye revealed nonvisualization of the gallbladder with multiple opaque stones. During the course of the infection there was a very limited increase in white blood cells, the maximum count being 8,350 with 38.5 per cent neutrophils two days before death. On Chart 2 are plotted the total neutrophil counts, percentages of nonfilament forms, and percentages of basophilic granule cells. This chart depicts an inadequate response dependent not upon the degree or duration of the infection but upon functional derangement of the bone marrow.

In the relapse phase of pernicious anemia there is decreased productivity of all myeloid elements, involving the leucopoietic and platelet forming as well as the erythropoietic tissues. Sturgis, Isaacs, and Smith<sup>13</sup> and Smithburn and Zervas,<sup>14</sup> among others, have shown that the presence of infection may inhibit the expected response to therapy in this disease. The bone marrow, consequently, may remain functionally incapable of reacting adequately to the infective process. Neither the degree nor the effort of the response is commensurate with the severity of the infection. In such a case the ordinary hemogram would be of little prognostic value.

CASE 3.—E. B. Pernicious anemia in beginning remission, acute infection (? encephalitis). An American born male of English-German descent, aged sixty-two years, and by occupation a photographer, entered the hospital complaining of weakness and persistent

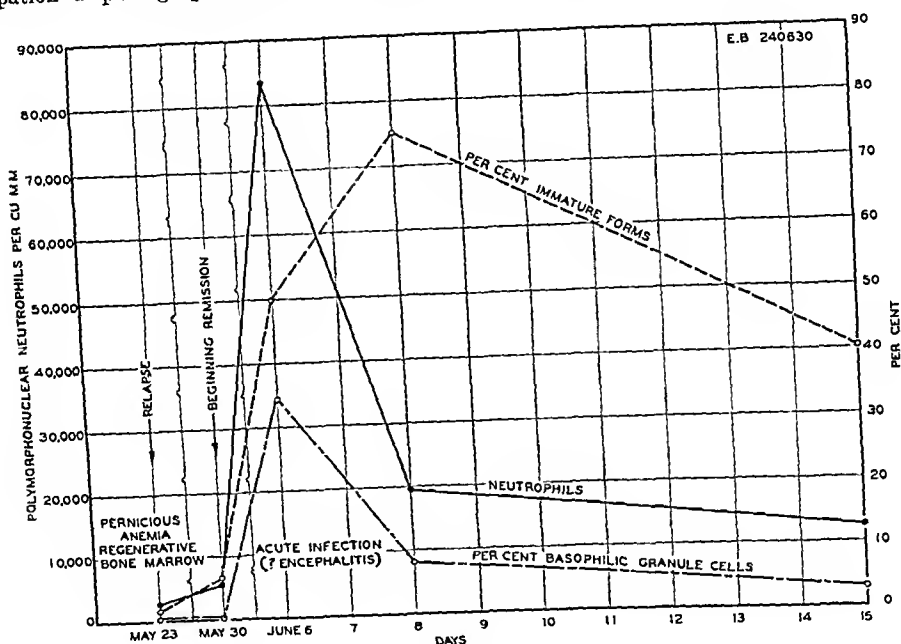


Chart 3.

numbness and tingling of the fingers and toes. His symptoms were of gradual onset and progression and had been first noticed about one year prior to his admission. Examination disclosed pallor and slight icterus, atrophy of the papillae of the skin, palpable liver and spleen, and impairment of vibratory sense and sense of motion and position in the lower extremities. Temperature was 99.8° F.; pulse, 88; respirations, 20. Ventriculin therapy was instituted and there occurred a typical hematologic response, accompanied by symptomatic improvement. However, thirteen days after admission the patient became acutely ill with moderate fever and marked leucocytosis. There was no localization of symptoms, extreme drowsiness and mental confusion being the only subjective manifestations. No objective signs were present; urinalysis, blood culture, and agglutination tests were negative; the lung fields were clear by roentgen examination; and lumbar puncture revealed only slightly increased pressure with normal spinal fluid. Acute encephalitis and subdiaphragmatic abscess were suggested respectively by neurologic and surgical consultants, and of the two conditions, the former, in the light of the subsequent course, appears more plausible. Within two weeks of its onset all evidences of the infection had disappeared, and remission of the anemia continued satisfactorily.

On admission the red blood cell count was 1,440,000 per c.mm.; hemoglobin, 36 per cent (Sahli); white blood cells, 4,350 per c.mm. Polymorphonuclear neutrophils were 60 per cent; eosinophils, 2 per cent; large lymphocytes, 11.5 per cent; small lymphocytes, 24.5 per cent; monocytes, 2 per cent. Achlorhydria of the gastric contents was present. Following ventriculin therapy the reticulocytes attained a maximum of 27 per cent. Subsequently, with the development of infection, the leucocyte count rose rapidly to 94,200 per c.mm. with a neutrophilic percentage of 88. Significant neutrophilic counts, percentages of immature forms, and percentages of basophilic granule cells for this case are given on Chart 3. It will be seen that the degree and effort of the bone marrow response are out of all proportion to the evidences of infection.

Fleming<sup>15</sup> and Heck and Watkins<sup>16</sup> have shown that the neutropenia and high percentage of segmented forms (shift to the right), which are commonly found in pernicious anemia in relapse, are replaced by a normal neutrophilic picture during therapeutically induced remission. In uncomplicated cases of pernicious anemia the increase in neutrophils and alteration of the nuclear shift, occurring in remission, are rarely of marked degree. However, if an infection develops during or soon after the period of active reticulocytosis there occurs an extraordinary neutrophilic response. Large numbers of nonfilament forms are discharged into the circulation and the immature cells may continue to predominate for some time after the infection has begun to subside. It appears that, in such cases, the bone marrow is in a hyperactive and supersensitive state, so that the stimulus of even a moderate infection elicits a maximal response.

CASE 4.—E. F. Postarsphenamine aplastic anemia and granulocytopenia; gangrenous stomatitis; latent syphilis. An American man, of forty-eight years, a construction engineer, was admitted complaining of weakness and bleeding from the gums. He was known to have had syphilis for twelve years and had received numerous courses of arsphenamine and neoarsphenamine therapy, together with other antisyphilitics. No clinical manifestations of his disease were present, but the Wassermann test persistently remained positive. Beginning six months before his admission he received 18 weekly injections of neoarsphenamine. After the fifteenth a rash developed over the legs; after the eighteenth, one month prior to admission, he experienced increasing weakness and shortness of breath. The treatment was discontinued, but he became progressively weaker, and noticed recurrent oozing of blood from the gums. He entered the hospital acutely ill; there was marked pallor of the skin; an exfoliative maculopapular eruption was present over the lower extremities and back; subretinal hemorrhages were seen; the pharynx was reddened with a considerable amount of mucoid exudate.

While in the hospital a tender swelling developed in the right lower alveolus. The lesion rapidly extended, and the temperature became markedly "septic" in type. There was little inflammatory reaction about the infective process in the mouth. Ultimately massive necrosis of the oral tissues developed, and on the twenty-sixth day of hospitalization the patient died.

The red blood cell count on admission was 1,300,000 per c.mm.; hemoglobin, 18 per cent (Sahli); white blood cells, 3,250 per c.mm. Polymorphonuclear neutrophils were 22 per cent; eosinophils, 1 per cent; large lymphocytes, 33 per cent; small lymphocytes, 40 per cent; monocytes, 4 per cent. The platelets were greatly reduced in number. During the period of acute infection the leucocyte count steadily declined, and on the day preceding death the count was 750 per c.mm. with 24 per cent neutrophils. The findings are recorded on Chart 4. The necropsy revealed syphilitic myocarditis, aortitis, and adenitis, gangrenous stomatitis and glossitis, multiple small internal hemorrhages, acute fibrinous pericarditis, and terminal lobular pneumonia. There was an almost complete fatty replacement of the cellular marrow of the sternum, ribs, and vertebrae.

A depressing effect upon the bone marrow of the arsenicals used in treating syphilis has long been recognized and the subject is well discussed by Farley.<sup>17</sup> Recently Loveman<sup>18</sup> has reviewed a number of reported cases of aplastic anemia following the administration of arsphenamine and related compounds. It is of interest that in the case presented here the percentage of basophilic granule cells parallels the clinical course of the infection, although no evidence of bone marrow response is obtained either from the total neutrophile counts or the determinations of the percentages of immature forms.

CASE 5.—E. E. Agranulocytic angina. A graduate nurse of twenty-six years was admitted to the otolaryngologic service complaining of severe sore throat of two days' duration.

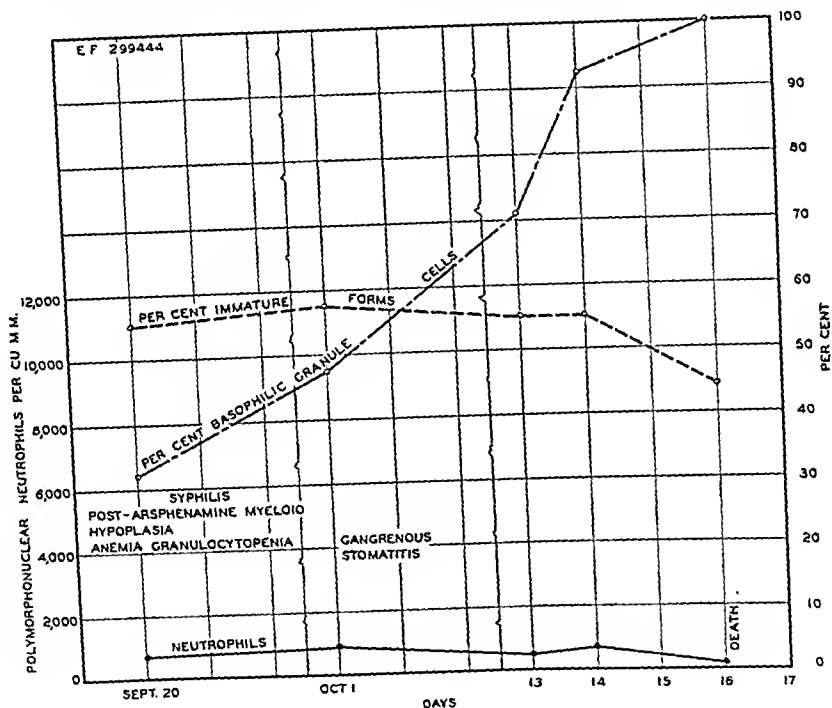


Chart 4.

Over a long period of time she had been accustomed to taking amidopyrine and barbitol for the relief of dysmenorrhea and chronic sinusitis. For five days preceding the onset of the sore throat she had taken 0.3 or 0.6 gm. of amidopyrine three times daily. On examination she appeared to be acutely ill. The pharynx and tonsils were intensely reddened with patches of gray exudate. Temperature was 99.2° F.; pulse, 108; respirations, 24. The temperature rapidly rose to over 103° and became "septic" in character. Leucopenia with marked reduction in the neutrophils was discovered and on the day following admission she was transferred to the medical service. Over a period of five days she was given 10 intramuscular injections each of 10 c.c. of 7 per cent pentnucleotide solution. During this time the infectious process in the throat progressed and areas of necrosis developed. After the fifth day of therapy there occurred a neutrophilic response and the local condition in the throat improved. However, signs of sepsis persisted and as the neutrophile count failed to increase appreciably after its initial rise administration of pentnucleotide was resumed. Ten days after admission the patient developed localized bronchopneumonia and pleurisy with effusion.

This was followed by empyema. She was transferred to the Department of Thoracic Surgery where open drainage was done. After a stormy convalescence she was able to leave the hospital fifty-four days after her admission.

On the day of transfer to the medical service the red blood cell count was 4,300,000 per c.mm.; hemoglobin, 70 per cent (Sahli); white blood cells, 800 per c.mm.; polymorphonuclear neutrophils, 8 per cent. Two days later the leucocyte count was 450 per c.mm., polymorphonuclear neutrophils, 27 per cent; large lymphocytes, 38 per cent; small lymphocytes, 31 per cent; monocytes, 4 per cent. On the fifth day of pentnucleotide therapy the white blood cell count was 3,050 of which 32 per cent were neutrophils. Subsequently, at the height of the pneumonic process, the count increased to a maximum of 22,000, 83 per cent neutrophils. On Chart 5 are plotted a few of the significant neutrophilic observations in this case. The intervening counts are, in general, in accord with the curves shown. It is not intended to give a detailed record of this patient's neutrophilic reaction during her illness, but rather

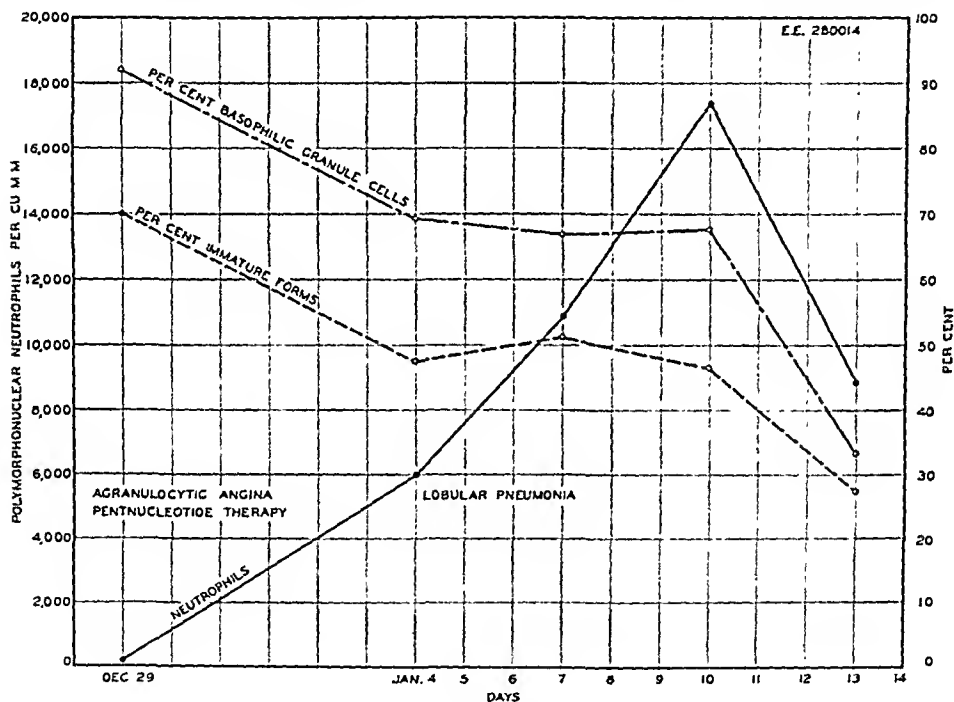


Chart 5.

to portray the character of the reaction. It should be observed that during the first period of the illness, coincident with the acute pharyngitis, there is a high percentage of basophilic granule cells without neutrophilia. Later, with the onset of pneumonia, the percentage of basophilic granulation again rises, but the factor interfering with response having been removed, there is a simultaneous increase in the total number of neutrophils.

It has long been known that chemicals possessing the benzene ring may depress bone marrow function, and Kracke,<sup>19</sup> from experimental evidence, had reason to believe that benzene compounds, in certain circumstances, exerted their action specifically on the leucopoietic elements of the bone marrow. Selling,<sup>20</sup> much earlier, was able to produce a leucopenia, without anemia, in rabbits by injecting benzene subcutaneously. However, it was not until the past year, following the reports of Madison and Squier<sup>21</sup> and of Watkins,<sup>22</sup> that a definite connection was established between severe neutropenia and the admin-



istration of certain drugs commonly employed in the practice of medicine. Of these the most conspicuous offender is amidopyrine. The action of this drug can only be surmised at present. The commonly accepted view is that in some individuals an idiosyncrasy to amidopyrine and related compounds exists with a resulting allergic reaction affecting the leucopoietic elements of the bone marrow. However, the high percentage of immature forms, in spite of the neutropenia, indicates that the bone marrow is at least capable of making an effort to respond. The explanation may lie, as suggested by Isaacs,<sup>23</sup> in the induction by the drug of an increased excretion of the neutrophiles into the alimentary tract.

CASE 6.—F. P. Typhoid fever. Extreme neutropenia. A young woman of twenty years, born in America of Polish ancestry, was transferred from the psychopathic service of the University Hospital to the Simpson Memorial Institute complaining of extreme malaise, feverishness, and lower abdominal pain. Six weeks previously she had been admitted to the psychopathic institution with a "psychiatric status typical of crude conversion hysteria in a feeble-minded individual." The medical history was not significant except that two months before her admission she contracted a severe upper respiratory infection with sore throat and fever. This illness lasted two weeks and left her weakened, depressed, and emotionally unstable. The nature of the treatment given at this time is not known, and no blood counts were made. The patient's course while on the psychopathic service was uneventful. Two weeks after her admission twelve carious teeth were extracted without sequelae. No drug therapy was given. On the day preceding her transfer she complained of generalized lower abdominal pain, was nauseated and vomited. The temperature was 103.6° F. Repeated blood counts revealed a progressive reduction of white cells affecting particularly the neutrophiles, and on the evening following the onset of symptoms she was transferred. It is probable that the acute illness was of longer duration than the records indicate, but because of the patient's unresponsiveness it was not recognized earlier. The temperatures of patients on the psychopathic service are not taken routinely. On examination the patient appeared acutely ill; facies apprehensive and dull; pharynx definitely reddened, but without exudate; lungs clear; heart negative; generalized moderate tenderness over the lower abdomen with increased muscle tonus, but no localized rigidity. Temperature was 106° F.; pulse, 88; respirations, 32. In spite of no known source of infection (no instances of typhoid fever had occurred in the psychopathic institution for a number of years), typhoid fever was suspected and its presence confirmed by blood culture, and serum agglutination tests. In addition to the usual measures of treatment 10 c.c. of 7 per cent pentnucleotide solution was given intramuscularly twice daily for five days, because of the persistent extreme neutropenia. Later intramuscular liver extract was used. Two transfusions each of 500 c.c. citrated whole blood were given. No symptomatic improvement nor significant increase in the white blood cell count followed these therapeutic measures. Eight days after recognition of the acute illness she died, following evidences of intestinal hemorrhage.

At the time of transfer to the Simpson Memorial Institute the red blood cell count was 2,530,000 per c.mm.; hemoglobin, 50 per cent (Sahl); white blood cells, 650 per c.mm.; polymorphonuclear neutrophiles, 20 per cent; eosinophiles, 0; basophiles, 0; large lymphocytes, 31 per cent; small lymphocytes, 47 per cent; monocytes, 2 per cent. A tabulation of the leucocyte counts, including those made before the onset of the acute illness, is given in Table II. The character of the neutrophile reaction, both before and during the acute illness, is portrayed in Chart 6. Throughout the rapidly fatal course of the disease, and in the face of an increasing degree of basophilic granulation, there is little change in the character of the bone marrow response.

The necropsy findings in this case include typhoid fever in stage corresponding to second or third week; large celled hyperplasia of intestinal lymphoid tissue with ulcers in lower ileum, cecum, and rectum; localized peritonitis; hyperplasia of retroperitoneal lymph nodes

This was followed by empyema. She was transferred to the Department of Thoracic Surgery where open drainage was done. After a stormy convalescence she was able to leave the hospital fifty-four days after her admission.

On the day of transfer to the medical service the red blood cell count was 4,300,000 per c.mm.; hemoglobin, 70 per cent (Sahli); white blood cells, 800 per c.mm.; polymorphonuclear neutrophils, 8 per cent. Two days later the leucocyte count was 450 per c.mm., polymorphonuclear neutrophils, 27 per cent; large lymphocytes, 38 per cent; small lymphocytes, 31 per cent; monocytes, 4 per cent. On the fifth day of pentnucleotide therapy the white blood cell count was 3,050 of which 32 per cent were neutrophils. Subsequently, at the height of the pneumonic process, the count increased to a maximum of 22,000, 83 per cent neutrophils. On Chart 5 are plotted a few of the significant neutrophil observations in this case. The intervening counts are, in general, in accord with the curves shown. It is not intended to give a detailed record of this patient's neutrophilic reaction during her illness, but rather

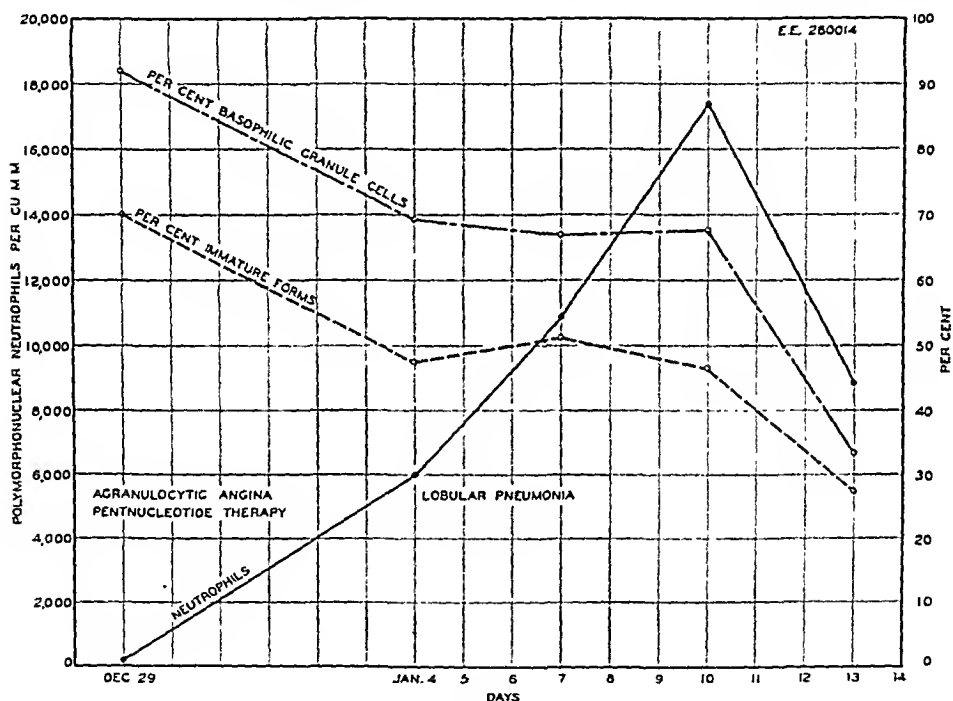


Chart 5.

to portray the character of the reaction. It should be observed that during the first period of the illness, coincident with the acute pharyngitis, there is a high percentage of basophilic granule cells without neutrophilia. Later, with the onset of pneumonia, the percentage of basophilic granulation again rises, but the factor interfering with response having been removed, there is a simultaneous increase in the total number of neutrophils.

It has long been known that chemicals possessing the benzene ring may depress bone marrow function, and Kracke,<sup>19</sup> from experimental evidence, had reason to believe that benzene compounds, in certain circumstances, exerted their action specifically on the leucopoietic elements of the bone marrow. Selling,<sup>20</sup> much earlier, was able to produce a leucopenia, without anemia, in rabbits by injecting benzene subcutaneously. However, it was not until the past year, following the reports of Madison and Squier<sup>21</sup> and of Watkins,<sup>22</sup> that a definite connection was established between severe neutropenia and the admin-

TABLE II  
LEUCOCYTE COUNTS ON CASE 6

DATE	W.B.C. PER C.M.M.	PER CENT							
		META- MYELO- CYTES	NONFILA- MENT NEUTRO- PHILES	FILA- MENT NEUTRO- PHILES	EOSINO- PHILES	BASO- PHILES	LARGE LYMPHO- CYTES	SMALL LYMPHO- CYTES	MONO- CYTES
5/10/33	6,100	0	8.0	56.0	1.0	1.0	18.0	11.0	5.0
5/16	4,900	0	12.0	41.0	2.0	0	25.0	18.0	2.0
6/13									
11 A.M.	5,100	0.5	13.5	14.0	0	2.0	38.0	23.0	9.0
6/13									
7 P.M.	600	1.0	12.5	11.5	0	0	36.0	23.0	16.0
6/13									
10 P.M.	650	2.0	11.0	7.0	0	0	41.0	26.0	13.0
6/14	650	4.5	33.0	16.5	0	0	25.0	16.0	5.0
6/15	1,000	1.5	33.0	16.5	0	0	28.0	18.0	3.0
6/16	950	2.5	18.0	16.5	0	0	45.0	17.0	1.0
6/17	700	1.0	20.0	18.0	0	0	47.0	11.0	2.0
6/18	500	5.0	24.0	23.0	0	0	35.0	12.0	1.0
6/19	1,350	5.0	22.0	15.0	0	0	44.0	14.0	0
6/20	650	6.0	17.0	12.0	0	0	46.0	18.0	1.0

No attempt has been made to formulate theories concerning the mechanism of the bone marrow response to infection, based on the clinical material presented here. The foregoing cases are reported as unusual types of myelocytic reaction to infection, and are illustrative of the ways in which primary disease of the bone marrow, or of the internal factors regulating hematopoiesis, may alter the nature of the response.

#### CONCLUSIONS

The response of the bone marrow to infection is dependent upon its pre-existing functional state, as well as upon the nature and severity of the infectious process.

The estimation of the number of neutrophils in the circulating blood and their classification into nonfilament and filament nuclear forms does not differentiate between the intrinsic and extrinsic factors involved in the response. The occurrence of basophilic granules in the cytoplasm of the neutrophils is, on the other hand, solely a result of the infection.

The total neutrophil count may be regarded as a measure of the degree of the bone marrow response, the percentage of nonfilament or immature forms as an indication of the effort of the response, and the percentage of neutrophils exhibiting basophilic granulation as a sign of the severity of the infection. By correlation of these data, obtained from successive blood examinations of the same individual, it is possible to ascertain the nature of the bone marrow response as well as the progress of the infection.

Six cases are reported of infection in the presence of various types of bone marrow dyscrasia which illustrate the manner in which this correlation is carried out. In these cases the percentage of neutrophils exhibiting cytoplasmic basophilic granulation gives a more accurate indication of the severity and the progress of the infection than either the total number of neutrophils per cubic millimeter or their degree of immaturity. Of the four fatal cases presented,

and spleen; focal necroses in liver; acute purulent bronchitis and bronchopneumonia. Microscopic examination of the marrow of the sternum, ribs, and vertebrae revealed a cellular marrow with a preponderance of erythroblastic elements.

This case of typhoid fever is remarkable because of the extreme neutropenia (130 neutrophils per c.mm.); its rapid development; and its occurrence during the first week of the disease. Thayer<sup>24</sup> found the lowest of 832 leucocyte counts made on uncomplicated cases of typhoid fever to be 1,000 cells per c.mm., the lowest neutrophilic percentage, 24.6, but as the leucocyte and differential counts are not correlated it is difficult to use his data for comparative purposes. Austin and Leopold<sup>25</sup> reported a case of more severe neutropenia than the one presented here, but in their patient the neutropenia developed in the third week of the disease and was accompanied by a great increase of lymphocytes, attaining 98 per cent, or 8,448 per c.mm.

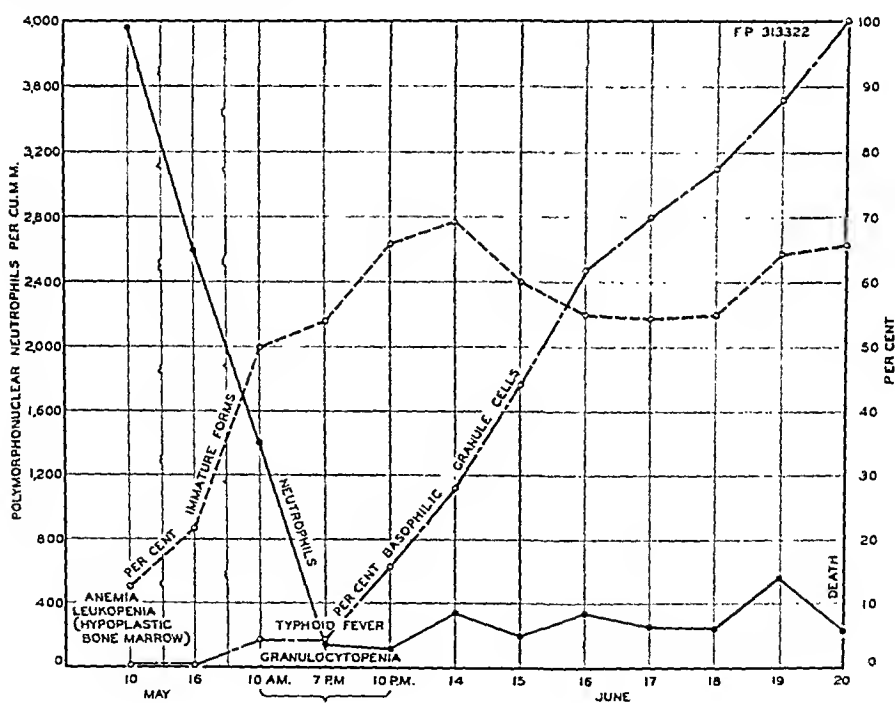


Chart 6.

The explanation of our patient's neutropenia probably lies in Schilling's<sup>26</sup> theory of leucopoietic paralysis by the toxemia of the infection with consequent "degenerative left nuclear shift." Whether the extreme effect was due to an especially virulent strain of *B. typhosus*, or to a primary functional defect of the bone marrow, it is impossible to say, but it is perhaps suggestive that the blood counts made before the acute illness revealed a reduction of all elements of bone marrow origin. There was a moderate degree of orthochromic anemia, a decrease in platelets, and a neutrophile count at or below the lower limit of normal. It may be justifiable to regard this case as one of constitutional hypoplasia of the bone marrow with unusual susceptibility to the toxic properties of the typhoid bacillus.

# ELLIPTICAL HUMAN ERYTHROCYTES\*

## OBSERVATIONS OF SIZE, VOLUME AND HEMOGLOBIN CONTENT

D. J. STEPHENS, M.D., AND A. J. TATELBAUM, M.D., ROCHESTER, N. Y.

THE occurrence of oval or elliptical erythrocytes in the blood of otherwise apparently normal individuals was first reported by Dresbach<sup>1</sup> in 1904. In a recent comprehensive review of the literature, McCarty<sup>2</sup> has collected reports of 79 instances of this abnormality of the red blood cells. The familial nature of the condition was suspected by Dresbach and has been amply confirmed, in particular, by studies of the families reported by Hunter and Adams<sup>3, 4</sup> and by Cheney.<sup>5</sup> It is the purpose of the present report to present hematologic observations of fifteen members of a family, eight of whom exhibited elliptical erythrocytes.

Rose I., a thirty-five-year-old Italian housewife, was admitted to the Gynecological Service of the Rochester Municipal Hospital in July, 1933, for dilatation and curettage because of excessive menstrual bleeding. There had been no symptoms of anemia or other blood disorder. Her father had died in Italy at the age of forty-five, after a five-year illness, during which his "blood turned to water." The patient had four healthy children. Her mother, sister, and three brothers were living and well.

Physical examination showed a well-developed and nourished woman who did not appear ill. The mucous membranes were of good color. There was no glandular enlargement. The heart and lungs were apparently normal. Blood pressure was 102 mm. Hg systolic, 60 diastolic. There was slight tenderness over the right side of the abdomen and over the liver edge, which was felt just below the costal margin. The spleen was not palpable. On pelvic examination the cervix uteri was found to be enlarged, lacerated and reddened, with widely patent external os. There were small external and internal hemorrhoids.

At the first examination the red blood cells numbered 5,100,000 per c.mm., white blood cells 9,000 per c.mm., hemoglobin 12.8 gm. per 100 c.c. of blood. The differential leucocyte formula was normal but in examining the blood film it was noted that the erythrocytes varied markedly in shape, with a large percentage of oval and elongated forms. Subsequent studies revealed that the variation in shape was constantly present in preparations of the patient's blood and in that of several members of her family. Reticulocyte counts varied between 0.2 and 1.2 per cent. In hypotonic saline, hemolysis of the red blood cells began at 0.38 per cent sodium chloride was complete at 0.26 per cent. In the control, hemolysis began at 0.40 per cent, was complete at 0.28 per cent. Other laboratory procedures, including urinalysis, stool examination, blood Wassermann reaction and cholecystogram, showed no abnormalities. Histologic examination of uterine curettings showed normal endometrium. For a few months some menorrhagia continued and there was occasionally a small amount of bleeding from hemorrhoids. These symptoms were accompanied by a slight decrease in hemoglobin and red blood cells.

\*From the Departments of Medicine and of Obstetrics and Gynecology of the University of Rochester School of Medicine and the Strong Memorial and the Rochester Municipal Hospitals.

Received for publication, May 22, 1934.

pathologic diagnoses were available on three, and so far as the methods of examination permitted, the conclusions arrived at by the blood studies were confirmed.

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variation in different films taken at the same time. In general, higher values were encountered in wet than in dry preparations. Although the majority of the "elliptical" cells were oval in outline, a few elongated, rodlike and irregularly shaped cells were observed in each preparation. The relative frequency of oval, elongated and irregularly shaped cells in both wet and dry preparations was approximately 8, 1.5, 1. All types of intermediate forms were encountered so that determinations of frequency of morphologic types were but rough estimates. In the wet preparations the oval and elongated cells were apparently biconcave ellipsoids. The ends of the cells were uniformly blunt and rounded. In general appearance they seemed similar to the poikilocytes commonly seen in the microcytic and macrocytic anemias. Crescent forms and filamentous processes characteristic of the cells in sickle-cell anemia were not seen. In staining characteristics and general morphologic appearance the oval and elongated cells seemed similar to the round cells. In Fig. 2 are shown photo-

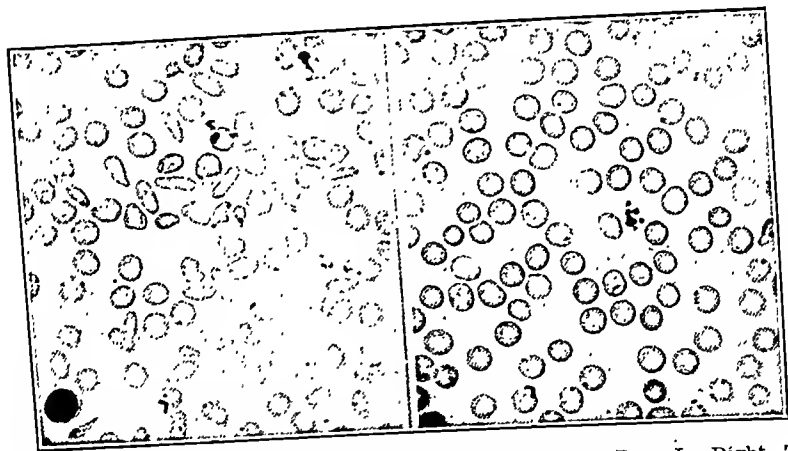


Fig. 2.—Photomicrographs of fixed stained blood films. Left, Rose I. Right, Tom G.

micrographs of fixed, stained smears of the blood of Rose I. and of her brother, Tom, whose red blood cells were apparently normal.

In individuals whose erythrocytes were classed as normal, an occasional oval cell was encountered but in these the degree of ellipticity was slight and no elongated or markedly irregular cells were encountered. The percentage of oval forms in wet and dry preparations of the blood of these was no greater than that frequently observed in other healthy persons, for the most part less than 1 per cent, in no case more than 5 per cent.

Detailed studies were made in the case of Rose I. for further identification of the type of the prevailing abnormality. Several wet preparations were observed over a period of twenty-four hours without change in the degree or frequency of the abnormal forms. The abnormality persisted unchanged after six hours in the ice box and in the incubator at a temperature of 56° C. The anomaly was present to approximately the same extent in wet, sealed preparations of capillary blood, oxalated blood, saline suspensions and in hanging drop

It was possible to secure blood for study from fifteen members of three generations of the family. Seven of these showed abnormalities in shape of the red blood cells similar to those observed in the patient (Fig. 1). Two of the younger children refused permission for venipuncture; in these, examinations were limited to capillary blood. Carrie R. refused permission to examine the blood of her four children. With the exception of Rose I., all of those examined were without complaints and were apparently healthy; none of them presented evidence of anemia, glandular enlargement, or splenomegaly.

Blood for fresh sealed preparations, fixed smears and reticulocyte counts was obtained from the lobe of the ear. The fixed smears were dried in air and stained with Wright's stain. Reticulocytes were enumerated in "wet" preparations stained with brilliant cresyl blue. The cell counts, hemoglobin estimations and volume of packed red blood cells were determined in venous blood, 5 c.c. of which were drawn into a bottle containing 0.01 gm. of dry potassium oxalate. The blood was thoroughly mixed before sampling. For the

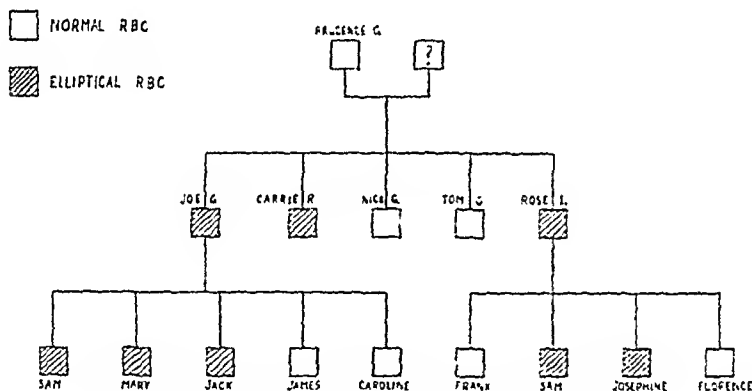


Fig. 1.—Familial distribution of elliptical erythrocytes.

estimation of the reticulocyte counts and the percentage of abnormally shaped erythrocytes in fresh sealed and fixed preparations, 1,000 erythrocytes were enumerated under the oil immersion lens. Hemoglobin was determined with a Sahli hemoglobinometer standardized by oxygen capacity. In order to reduce the error in estimating the number of red blood cells, the average of two counts was taken for each determination. Pipettes and counting chambers certified by the Bureau of Standards were used. Volume of packed red cells was determined by means of the Wintrobe hematocrit tube and corrected for shrinkage due to the anticoagulant. The mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were calculated according to the formulas given by Wintrobe.<sup>6</sup> In estimating the diameter of erythrocytes in fixed, stained smears, 500 cells were measured in two dimensions by means of an ocular micrometer which had been calibrated for microscopic tube length and working conditions.

In the blood of the eight individuals with elliptical erythrocytes, the percentage of the abnormally shaped cells varied from 20 to 56 per cent. They were present in greatest numbers in the blood of Rose I. There was considerable



in hemoglobin and volume of packed red cells. In each instance the number of reticulocytes was within normal limits. There was no increase in the icterus index. Except for a mild neutrophilic leucocytosis in three of the normals, there were no abnormalities in the total white blood cell or differential counts. In fixed smears the platelets appeared to be normal in number and structure.

In examining the wet and fixed blood films of various members of the family, difference in the size of the erythrocytes in the two groups was apparent. Two dimensional measurements of the red blood cells were made in fixed, stained cover slip preparations of the blood of four individuals with elliptical cells and in four subjects of similar age with normal cells. The results of these studies are shown in Table II. In each of the four normals, the figures are in agreement with the values given by Price-Jones<sup>8</sup> and by Wischniewsky<sup>9</sup> for the mean diameter of normal human erythrocytes in fixed, stained smears. In con-

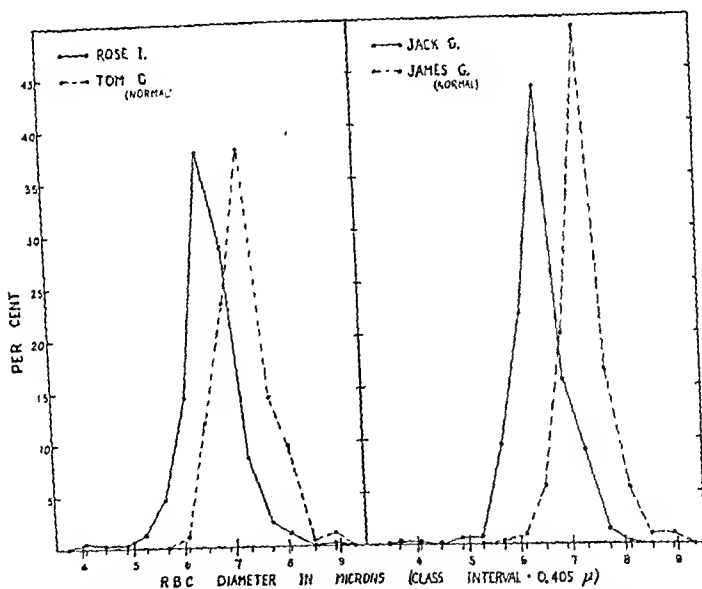


Fig. 3.—Frequency distribution curves of red blood cell diameters in two individuals with elliptical erythrocytes and in two subjects with red blood cells of normal shape.

trast, the round cells of those individuals exhibiting a large number of elliptical cells were consistently and significantly smaller, averaging 6.52 microns in diameter. Frequency distribution curves of round cell diameters of two members of each group are shown in Fig. 3. The calculated average diameter of the elliptical cells was about the same as the observed diameter of the round cells. Measurements of the length and width of the oval and elongated cells were scattered over a wide range, as indicated in the table. However, the majority of the abnormally shaped cells were oval in outline and the variation in the average dimensions of these cells was of approximately the same magnitude as for the round cells. In considering the above results it should be noted that drying and staining causes an appreciable shrinkage of erythrocytes, so that cells in fixed, stained preparations are 1 to 1.5 microns smaller than the same cells in plasma, serum, or isotonic saline.

preparations. On three occasions the cells were studied before and after twenty minutes' exposure to carbon dioxide according to the technic described by Hahn and Gillespie.<sup>7</sup> No change was noted in the shape of the cells or in the frequency of the abnormal forms. The appearance of the cells, their behavior under the above conditions, the familial distribution and the lack of significant anemia identified the anomaly as that which has been described as "elliptical human erythrocytes" or "ovalocytosis."

Quantitative hematologic observations are summarized in Table I. In affected individuals, the erythrocytes were uniformly above 5 million per c.mm., but the hemoglobin was not correspondingly increased. In spite of the apparent polycythemia the volume of packed red blood cells was within normal limits, so that in each instance the mean corpuscular volume and mean corpuscular content of hemoglobin were definitely decreased. However, the degree of saturation of corpuscular mass with hemoglobin (MCHC) was not significantly lowered. In those members of the family whose red blood cells were considered normal, corpuscular volume and hemoglobin content were well within the generally accepted normal range. In two of the latter group, the erythrocytes numbered more than 5 million per c.mm., but in these there was a corresponding increase

TABLE I  
HEMATOLOGIC OBSERVATIONS OF FIFTEEN MEMBERS OF THE FAMILY OF ROSE I.

SUBJECT	AGE	DATE	ELLIPTICAL CELLS	HEMOGLOBIN GRAMS PER 100 C.C.	RED BLOOD CELLS MILLIONS PER C.M.M.	V.P.R.* (COR.) C.C. PER 100 C.C.	M.C.V.*	M.C.H.*	M.C.H.C.*	RETICULOCYTES PER CENT	WHITE BLOOD CELLS PER C.M.M.
Rose I.	35	8/10	+	13.05	5.97	40.9	68.4	21.7	31.9		9,200
		8/17	+	12.3	5.91	39.2	66.4	20.8	31.4	1.2	8,850
		2/16	+	11.3	5.46	36.0	65.9	20.7	31.4	0.2	
Sam I.	9	8/17	+	12.75	7.15	38.0	53.1	17.8	33.5	1.0	8,100
Josephine I.	8	8/17	+	12.15	5.51	36.0	65.4	22.1	33.8	1.0	9,250
Joe G.	43	8/10	+	15.5	7.27	46.8	64.3	21.3	33.1	0.8	7,650
		8/24	+	15.0	8.06	46.8	58.1	18.6	32.1	1.2	8,000
Sam G.	17	8/24	+	13.8	6.80	43.1	63.4	20.3	32.0	0.2	9,100
Mary G.	11	8/24	+	13.2	7.98	40.3	50.5	16.5	32.8	0.6	8,500
Jack G.	10	8/24	+	12.6	5.72	38.1	66.6	22.0	33.1	0.8	8,400
Carrie R.	37	9/27	+	15.0	5.36	40.9	76.2	28.0	36.7	1.0	6,800
Average			+	13.3	6.47	40.6	63.5	20.9	32.9	0.8	
Prudence G.	67	9/27	0	15.45	4.94	43.6	88.1	31.2	35.4	0.6	13,500
Frank I.	10	8/17	0	14.85	4.82	42.5	88.1	30.8	35.0	0.6	9,400
Florence I.	5	8/17	0								
James G.	8	8/24	0	12.45	4.33	36.0	83.1	28.8	34.6	0.2	12,500
Caroline G.	7	8/24	0	14.25	4.90			29.1		1.0	19,500
Nick G.	31	8/10	0	18.1	6.05	50.1	83.0	30.0	36.1	0.2	7,700
		3/25	0	17.7	6.40	52.0	81.2	27.7	34.0	0.8	8,000
Tom G.	28	8/10	0	17.8	5.65	49.0	86.8	31.5	36.3	0.4	5,200
		3/25	0	16.6	5.42	47.9	88.4	30.6	34.6	0.6	5,500
Average			0	15.9	5.31	45.9	85.5	30.0	35.1	0.6	

\*V. P. R., volume packed red cells.

M. C. V., mean corpuscular volume in cubic microns.

M. C. H., mean corpuscular hemoglobin in micromicrograms.

M. C. H. C., mean corpuscular hemoglobin concentration in per cent.

The nature and etiology of the observed abnormality have been the subject of much discussion. Several observations are on record of studies of bone marrow obtained at autopsy or by sternal puncture.<sup>8, 10, 11, 12, 13</sup> Red blood cell progenitors have uniformly been round in shape, although mature erythrocytes in sections of bone marrow and other tissues have appeared similar in outline to those observed in preparations of circulating blood. Nucleated red blood cells have not been observed in the peripheral circulation. During the present study a large proportion of the reticulocytes observed in the blood of affected individuals were definitely oval in outline but the degree of ellipticity was not marked. No elongated or grossly irregular reticulocytes were seen. The abnormality in shape persists in various isotonic solutions, in surviving cells in hypotonic saline, in the serum of normal individuals and in the presence of the usual anticoagulants. The percentage of abnormally shaped cells in cases reported in the literature has varied from 10 per cent to more than 90 per cent; there are no recorded observations of 100 per cent elliptical cells. In the same patient, considerable variation in frequency and degree of poikilocytosis is encountered in individual preparations taken at the same or different times. In general the anomaly is more marked in wet than in dry preparations. Terry, Hollingsworth and Eugenio<sup>10</sup> cite experiments which indicate that the temperature and the degree of trauma to which the cells are subjected in preparing them for examination may influence their shape, and that the oval cells are heavier and more resistant to hypotonic saline than are the round erythrocytes. The authors and others<sup>2</sup> have been unable to confirm these observations. Two dimensional measurements indicate that the abnormally shaped cells are of approximately the same size as the round cells and that the variability of distribution of cell size is approximately normal. In both wet and fixed preparations the normal and abnormal cells appear to be similar morphologically except for the difference in shape. It is probable that in affected individuals the potentiality for the deformity is possessed by all the mature erythrocytes, perhaps to a lesser degree by the nonnucleated reticulocytes. The factors which determine the observed shape of individual cells have not as yet been clearly defined.

In the authors' cases, each of the affected individuals showed a definite decrease in mean corpuscular volume and in mean corpuscular hemoglobin content. Two dimensional measurements of the erythrocytes in fixed, stained smears indicated that both the round and abnormally shaped cells contributed to the decrease in mean cell volume. The decrease in average cell size was partially, in some instances completely, compensated for by an increase in the red blood cell count so that the average volume of packed red blood cells was approximately normal. Except for a shift toward the smaller cell groups, the frequency distribution curves of cell size was approximately the same in the affected as in the normal individuals (Fig. 3). This may be contrasted with the increase in variability of cell size and the marked broadening and flattening of the frequency distribution curve, which usually accompany the poikilocytosis of the macrocytic and microcytic anemias.<sup>8</sup> A similar increase in variability of cell size may occur in those individuals with elliptical erythrocytes who also present anemia.

TABLE II  
TWO DIMENSIONAL MEASUREMENTS, IN MICRONS, OF RED BLOOD CELLS OF EIGHT MEMBERS OF THE FAMILY

SUBJECT	ROUND CELLS				ELLIPTICAL CELLS						CALCULATED MEAN DIAMETER
	MEAN DIAMETER	STANDARD DEVIATION	COEFFICIENT OF VARIATION	PROBABLE ERROR	UPPER LENGTH	LOWER LENGTH	UPPER WIDTH	LOWER WIDTH	AVERAGE LENGTH	AVERAGE WIDTH	
Rose I.	6.59	0.554	8.4%	0.0186	12.16	5.67	7.29	1.62	8.15	5.38	6.76
Joe G.	6.73	0.234	3.5%	0.0075	12.16	4.86	7.29	1.62	7.32	5.44	6.38
Jack G.	6.44	0.515	8.1%	0.0163	11.35	6.48	7.29	3.24	8.06	5.29	6.67
Sam I.	6.50	0.404	6.5%	0.0129	9.72	5.67	7.29	4.05	8.72	5.02	6.87
Average	6.56	0.427	6.6%	0.0141	11.30	5.67	7.29	2.63	8.06	5.28	6.67
Nick G.	7.31	0.620	8.4%	0.0188	The calculated mean diameter of elliptical cells is taken as the mean of the average length and the average width.						
Tom G.	7.25	0.510	6.9%	0.0154							
James G.	7.28	0.423	5.8%	0.0129							
Frank I.	7.24	0.546	7.5%	0.0168							
Average	7.27	0.537	7.1%	0.0159							

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## THE COLLOIDAL GOLD REACTION IN 500 CASES OF NEUROSYPHILIS\*

WILLIAM C. MENNINGER, M.D., TOPEKA, KAN., AND  
LEON BROMBERG, M.D., CHICAGO, ILL.

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THE following brief report concerns the colloidal gold reaction in 500 cases of neurosyphilis, with particular attention to the variations in the curve in the different clinical types of the disease. The diagnosis in every instance was based primarily on the clinical picture, and was supported by a positive cerebrospinal fluid Wassermann test in all cases. The 500 patients were unselected and many had had varying amounts of antisyphilitic treatment before coming to the clinic.

The colloidal gold reaction in neurosyphilis has been the subject of a great many investigations, both with regard to its diagnostic and prognostic value. The most recent comprehensive study was made by Novy<sup>1</sup> who analyzed the colloidal gold reaction in the cerebrospinal fluids of 500 cases of neurosyphilis. As a control the author used 200 cases of systemic syphilis without central nervous system involvement. Novy concluded: that syphilis without central nervous system invasion may frequently produce some change in the colloidal gold reaction; that no one type of neurosyphilis presents a constant curve; that first zone curves, even when pronounced, do not necessarily indicate general paralysis; that a middle zone curve is not of diagnostic significance since it occurs frequently in all types of neurosyphilis; and that there is not sufficient uniformity in the changes in general to merit prognostic significance.

The results of the present investigation are shown in Table I. The colloidal gold tests were all carried out in the same laboratory according to the standard technique, using a gold sol prepared by the method of Levine,<sup>2</sup> the

\*Received for publication, May 28, 1934.

In previously reported cases, the red blood cells have been in excess of 5 million per c.mm. in only a few instances. In the majority there have been varying degrees of anemia, with the color index usually below one. One would expect the latter finding to be accompanied by a decrease rather than an increase in the mean corpuscular volume. The available literature contains few observations of the size of elliptical human erythrocytes. In Dresbach's case,<sup>1</sup> average size of the elliptical cells was 10.3 by 4.1 microns; extremes in length were 10.7 and 8.5 microns, in width 4.8 and 3.9 microns. The cells of Bishop's patient<sup>14</sup> were measured in dry films; the round cells averaged 8.5 microns in diameter, the elliptical cells varied in length from 17 to 8.2 microns, in width from 8.0 to 3.2, averaging 13 by 5 microns in size. Sydenstricker<sup>15</sup> recorded variations of from 13.0 to 8.5 microns in length, 4 to 6 microns in width, with the round cells 8 to 8.5 microns in diameter. Huck and Bigalow<sup>16</sup> measured the cells of one of their patients in both wet and dry preparations. In the former the round cells varied from 6 to 11 microns in diameter, in the latter from 5 to 9 microns; in this patient the red blood cell count was well over 5 million per c.mm. and the color index was about 0.8. In a patient with anemia and color index less than one, Hunter and Adams<sup>3</sup> found the round cells to have a mean diameter of 9 microns. In two of McCarty's patients, both with anemia, the volume index was 1.23 and 1.50.<sup>2</sup> In the latter, the mean diameter of the round cells measured in wet preparations was only 8.0 microns, in spite of the high volume index; in this patient the oval cells measured 13.3 by 5.78 by 2.89 microns. The variations in recorded observations are difficult to interpret because of failure in some instances to indicate the method used or the conditions under which the cells were measured. Observations of additional cases under controlled conditions must be made before it can be said with certainty that definite abnormalities in size and hemoglobin content are characteristic of elliptical human erythrocytes.

#### SUMMARY

1. Hematologic studies are presented of fifteen members of an Italian family, eight of whom exhibited elliptical erythrocytes.

2. In each of the affected individuals, there was an increase in the number of red blood cells per cubic millimeter with definite decrease in mean corpuscular volume and mean corpuscular hemoglobin. In general the number of red blood cells was inversely proportional to the mean cell volume and hemoglobin content.

3. Two dimensional measurements of cells in fixed, stained smears indicated that the decrease in size involved the round cells and the abnormally shaped cells to approximately the same extent, without increase in the variability of distribution of cell size.

4. In the unaffected members of the family the erythrocytes were normal in shape, size, volume, and hemoglobin content.

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## CONCLUSION

1. An analysis of the colloidal gold reaction in the cerebrospinal fluids of 500 patients suffering from neurosyphilis showed no constant correspondence between the pattern and degree of precipitation and the type of central nervous system involvement.

2. The colloidal gold curve, while of considerable confirmatory value in the presence of clinical signs of general paresis, is in itself not diagnostic.

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## A COMPARISON OF VARIOUS CONCENTRATIONS OF SODIUM OXALATE SOLUTION WITH HEPARIN FOR THE DETERMINATION OF PACKED CELL VOLUME\*

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ORVILLE S. WALTERS, PH.D., ST. LOUIS, MO.,  
AND JAMES W. MAY, LAWRENCE, KAN.

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THE utilization of standard centrifuge tubes for the routine determination of packed cell volume appears to have originated with Keith, Rowntree and Geraghty (1915) in connection with blood volume studies. They employed dry oxalate as an antieoagulant. The use of an isotonic oxalate solution to prevent shrinkage of cells was suggested by Hooper, Smith, Belt, and Whipple (1920) who introduced 1.6 per cent sodium oxalate solution for this purpose.

The choice of this concentration of sodium oxalate was based, by these investigators, both on physicochemical relations and biologic tests. In the first instance, they ascertained from chemical tables that a 1.6 or a 1.7 per cent solution of sodium oxalate would be approximately isotonic with a 0.95 per cent solution of sodium chloride if the dissociations of sodium and potassium oxalates were not far different.

The biologic tests consisted of three experiments. Ten cubic centimeters of defibrinated dog's blood were placed in each of eight tubes. Seven of these contained 2 c.c. quantities of sodium oxalate in concentrations varying from 1.0 to 1.6 per cent. The eighth tube contained no antieoagulant. Two such sets of tubes were centrifuged at 3,000 r.p.m. for one-half hour in the first experiment. The second experiment was identical except that blood from a different dog was used. Comparison of the single control tube with the others in each of these four series showed that the packed cell volume in the tube containing 1.6 per cent oxalate solution was closest to control value.

In the third experiment, blood from a dog in peptone shock was employed. Four tubes of this blood were centrifuged. Three contained 2 c.c.

\*From the Department of Physiology, University of Kansas.  
Received for publication, June 18, 1934.

director of the laboratory. For convenience, we have grouped the curves into five classes: (1) a low first zone curve in which the change was limited to the first zone of the curve, and maximum color change did not exceed the value indicated by the number "3"; (2) a strong first zone reaction, in which the maximum was "4" in at least one tube; (3) the so-called "paretic" reaction, in which the change was in the first zone, and reached a maximum of "5," usually in two or more tubes; (4) a low second zone curve, in which the change was limited to the second zone and the maximum color change was "3" or less; (5) a strong second zone reaction with the maximum color change reaching "4" or "5."

TABLE I

COLLOIDAL GOLD REACTION	PARESIS		TABO-PARESIS		MENINGO-VASCULAR NEUROSYPHILIS		TABES DORSALIS		ASYMPTOMATIC NEUROSYPHILIS	
	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT	NO. OF CASES	PER CENT
Negative	0	0	0	0	1	7.7	4	3.0	14	4.7
(1) Low zone I	3	7.9	2	10.5	2	15.4	31	23.3	53	17.8
(2) Strong zone I	3	7.9	3	15.8	5	38.4	25	18.5	65	21.9
(3) "Paretic"	27	71.1	11	57.8	4	30.8	52	39.0	86	28.9
(4) Low zone II	0	0	2	10.5	0	0	8	6.1	43	14.5
(5) Strong zone II	3	7.9	0	0	0	0	11	8.3	33	11.1
Not done	2	5.2	1	5.2	1	7.7	2	1.5	3	1.0
Totals	38	100.0	19	100.0	13	100.0	133	100.0	297	100.0

*General Paresis.*—From Table I it is evident that 71.1 per cent of the 38 cases of paresis presented the maximum first zone reaction, often regarded in the past as specific for this type of neurosyphilis. Only 65 per cent of the 100 cases of general paralysis reported by Novy<sup>1</sup> showed the typical maximum first zone curve.

*Taboparesis.*—As expected, this group showed the next highest percentage (57.8 per cent) of cases with the maximum first zone curve, as compared with 32 per cent in Novy's series. In our cases only two (10.5 per cent) showed a change limited to the second zone and, in both instances, the changes were slight.

*Meningovascular Neurosyphilis.*—This series of only 13 cases was too small to allow definite conclusions. All fluids in this series showed a deviation of the colloidal gold curve in the first zone.

*Tabes Dorsalis.*—The significant finding in this group of 133 cases was the large percentage (39 per cent) with a maximum first zone, or so-called "paretic" type of curve. (Only 11 per cent of Novy's 100 cases of tabes dorsalis showed this type of reaction.) Four of our cases showed no change in the colloidal gold reaction despite very definite clinical evidence of tabes, as well as a positive cerebrospinal fluid Wassermann test.

*Asymptomatic Neurosyphilis.*—In this large series of 297 cases, we found 28.9 per cent showing the so-called "paretic" type of curve, and 68.6 per cent showing some change limited to the first zone as compared to 25.6 per cent showing changes limited to the second zone. Fourteen cases (4.7 per cent) showed no change in the colloidal gold reaction.



Two centrifuges were used in the experiments, an International 1SB and an International 1C. Each has a working radius of 9 cm., but the former was run at a speed of 3,600 r.p.m. while the latter was rotated at 2,800 r.p.m.

In all, 50 experiments were conducted. A total of 800 tubes were centrifuged and read, less a few which broke in the machines. The concentrations of sodium oxalate compared were 1.35 per cent, 1.4 per cent, 1.5 per cent, 1.6 per cent and 1.7 per cent. The accuracy of the solutions was checked by a titrimetric method.

## RESULTS

The average figures for the experiments using 1.4, 1.5, and 1.6 per cent oxalate solutions are shown in Tables I, II, and III. In Table IV, the comparative data for all concentrations of oxalate compared are summarized. From this table it is apparent that 1.35 and 1.4 per cent solutions of oxalate are definitely hypotonic, since the packed cell volumes are greater than those with heparin alone. The 1.6 per cent and 1.7 per cent solutions are definitely hypertonic, giving packed cell volumes lower than heparin alone. The packed

TABLE I  
COMPARATIVE PACKED CELL VOLUMES  
Heparin and 1.4 Per Cent Sodium Oxalate Solution

EXPERIMENT	HEPARIN-OXALATE		HEPARIN ONLY	
	PACKED CELL VOLUME	STANDARD DEVIATION	PACKED CELL VOLUME	STANDARD DEVIATION
16	45.1	0.755	44.6	0.464
17	37.7	0.850	38.5	0.529
18	42.5	1.030	41.6	0.552
19	38.1	0.775	37.9	0.458
20	36.9	0.446	36.7	0.847
21	36.3	0.421	37.2	0.815
22	31.5	0.335	31.4	0.731
23	41.9	0.187	38.0	0.423
24	36.0	0.595	36.2	0.619
25	34.5	0.414	33.6	0.432
Mean	38.05	0.5808	37.57	0.5870

TABLE II  
COMPARATIVE PACKED CELL VOLUMES  
Heparin and 1.5 Per Cent Sodium Oxalate Solution

EXPERIMENT	HEPARIN-OXALATE		HEPARIN ONLY	
	PACKED CELL VOLUME	STANDARD DEVIATION	PACKED CELL VOLUME	STANDARD DEVIATION
26	34.8	0.609	34.5	0.581
27	39.3	0.704	39.8	0.470
28	38.5	0.658	38.9	0.501
29	34.2	0.323	33.7	0.595
30	34.8	0.494	34.7	0.261
31	35.4	0.863	36.2	0.501
32	36.6	0.480	35.8	0.335
33	35.4	0.664	35.5	0.446
34	34.9	0.476	34.9	0.426
35	28.9	0.577	28.5	0.414
Mean	35.28	0.5853	35.25	0.4530

respectively of 1.0 per cent, 1.4 per cent, and 1.7 per cent sodium oxalate solution, while the fourth contained no anticoagulant. In this experiment, the 1.4 per cent solution was hypotonic and the 1.7 per cent hypertonic. By interpolation, the point of isotonicity was considered to be 1.5 per cent.

From these tests and the physicochemical data, a 1.6 per cent solution was adopted by Hooper and others as being approximately isotonic with dog's blood. This concentration was subsequently accepted by Haden (1923) and various other investigators for use on human blood.

The objections are raised by Van Allen (1925) to the work described above that in the defibrination of blood, corpuscles would necessarily be lost and also that the average size of erythrocytes is reduced in peptone shock. Either factor would tend to suggest the 1.6 per cent concentration as being too high. Van Allen states that a 1.5 per cent solution of oxalate is required for ascertaining the packed cell volume of rabbit's blood by the Hooper method. With his capillary hematocrit, however, Van Allen recommends 1.3 per cent oxalate as being in best osmotic agreement with rabbit's blood.

Smith and Prest (1932) adopted 1.3 per cent sodium oxalate in their studies on human blood after comparison of various concentrations with the Van Allen hematocrit. They report that this percentage gives values very close to those for whole blood.

In the experiments conducted by Haden (1930) on human blood with 12 c.c. centrifuge tubes, 1.4 per cent oxalate gave packed cell volumes closer to the values obtained with hirudin and heparin than any other concentration. This per cent of oxalate has since been generally employed for routine use with human blood.

The present study was made to determine by an adequate series of comparisons the concentration of sodium oxalate which yields packed cell volume values for dog's blood agreeing most perfectly with those in which heparin is employed as the anticoagulant.

#### METHODS

Two healthy 35 kg. dogs were used to furnish the necessary blood. They were alternated to avoid any pronounced anemia. In each experiment, a 100 c.c. quantity of blood was withdrawn from the external jugular vein into a large syringe containing 1 c.c. of 0.35 per cent heparin in 0.9 per cent sodium chloride solution, an amount alone sufficient to prevent coagulation. The blood and heparin were thoroughly mixed in the syringe, then exactly 40 c.c. were run into a graduate cylinder containing 8 c.c. of the sodium oxalate solution to be compared. This was mixed carefully by inversion and immediately divided equally among eight 12 c.c. graduated centrifuge tubes.

The remainder of the heparinized blood was divided among a second series of 12 c.c. tubes. The two sets of tubes (16 in all) were then centrifuged for one hour, removed and read, and returned for additional rotation until the volume remained constant. After correction for the oxalate added, the percentages were calculated and recorded. All readings were made by the same individual.

The limit of error thus determined for the standard tube method in the present study averages 0.679 c.c. (standard deviation) or 1.71 per cent (coefficient of variation) for heparin-oxalate tubes and 0.576 c.c. or 1.45 per cent for heparin alone. As this difference in variation became apparent in the course of the study, the procedure was varied slightly to ascertain the effect of different amounts of blood, since a larger quantity was being used in the heparin series (7.5 c.c. in each tube) than in the oxalate series (5 c.c. in each tube).

The results are shown in Table V. A much smaller error, approximately 1 per cent, was found when the amount of blood in the oxalate tubes was increased to 10 c.c. Likewise, the limit of error of the heparin tubes increased from 1.43 to 1.57 per cent when a smaller quantity of blood was employed.

As a further modification in the method, both 15 c.c. and 12 c.c. tubes were used. The comparative data are given in Table VI. It will be seen that the error is slightly greater in the 15 c.c. than in the 12 c.c. tubes.

TABLE VI  
EFFECT OF SIZE OF TUBE USED

SIZE OF TUBE	NO. EXPER.	HEPARIN-OXALATE (5 C.C. BLOOD)			HEPARIN (7.5 C.C. BLOOD)		
		NO. TUBES	AVER. STAND. DEV.	COEFF. OF VARIATION (PER CENT)	NO. TUBES	AVER. STAND. DEV.	COEFF. OF VARIATION (PER CENT)
15 c.c.	20	160	0.7384	1.860	159	0.6059	1.526
12 c.c.	24	191	0.7010	1.766	189	0.5473	1.379

A careful check was kept of the number of tubes in which packing was complete at the end of one hour. Of the nearly 800 tubes, 97.5 per cent showed no change in volume after one hour of rotation.

#### SUMMARY

1. A series of 800 comparative packed cell volume determinations made by the standard centrifuge tube method on fifty 100 c.c. samples of dog's blood demonstrates that a 1.5 per cent solution of sodium oxalate gives values practically identical with those obtained with heparin.

2. The minimum error found for the standard tube method was approximately 1 per cent, obtained when 10 c.c. quantities of blood were employed. The error is proportionately greater when smaller quantities of blood are used. It is slightly less with 12 c.c. than with 15 c.c. tubes.

3. Packing was complete after one hour of rotation in 97.5 per cent of cases.

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cell volume values obtained with 1.5 per cent sodium oxalate solution, however, are practically identical with those in which heparin alone was employed as the anticoagulant.

*Error of the Method.*—The coefficient of variation derived from simultaneous determinations on the same sample of blood has been used as a measure of the error inherent in the method. This apparently has not been ascertained for the standard tube method, although Wintrobe (1930) found variations no greater than 0.5 per cent with his 3 mm. bore hematocrit tubes.

TABLE III  
COMPARATIVE PACKED CELL VOLUMES  
Heparin and 1.6 Per Cent Sodium Oxalate Solution

EXPERIMENT	HEPARIN-OXALATE		HEPARIN ONLY	
	PACKED CELL VOLUME	STANDARD DEVIATION	PACKED CELL VOLUME	STANDARD DEVIATION
36	32.6	1.082	32.5	0.466
37	37.9	0.476	39.0	0.576
38	35.4	0.596	36.2	0.550
39	32.1	0.385	32.3	0.256
40	32.5	1.045	32.6	0.737
41	29.5	0.289	30.3	0.570
42	46.3	0.570	46.4	0.514
43	46.4	0.765	47.8	0.975
44	44.8	0.926	44.9	0.499
45	43.3	0.280	44.6	0.699
Mean	38.08	0.6414	38.66	0.5842

TABLE IV

SUMMARY OF ALL EXPERIMENTS SHOWING COMPARISON OF VARIOUS SODIUM OXALATE SOLUTIONS WITH HEPARIN

NO. EXPER.	CONC. OF OXALATE SOLUTION	HEPARIN-OXALATE			HEPARIN ONLY			DIFFERENCE OF OXALATE (PACKED CELL VOLUME)
		NO. OF TUBES	AVER. PACKED CELL VOLUME	AVER. STANDARD DEVIATION	NO. OF TUBES	AVER. PACKED CELL VOLUME	AVER. STANDARD DEVIATION	
15	1.35	120	43.86	0.7687	120	43.19	0.5735	+0.67
10	1.40	80	38.05	0.5808	77	37.57	0.5870	+0.48
10	1.50	80	35.28	0.5853	76	35.25	0.4530	+0.03
10	1.60	79	38.08	0.6414	80	38.66	0.5842	-0.58
5	1.70	40	43.26	0.8136	40	43.80	0.7938	-0.54
Mean			39.77	0.6789		39.63	0.5763	

TABLE V  
EFFECT OF QUANTITY OF BLOOD USED

QUANTITY OF BLOOD	HEPARIN-OXALATE				HEPARIN			
	NO. EXPER.	NO. TUBES	AVER. STAND. DEV. (C.C.)	AVER. COEFF. OF VAR. (PER CENT)	NO. EXPER.	NO. TUBES	AVER. STAND. DEV. (C.C.)	AVER. COEFF. OF VAR. (PER CENT)
10.0 c.c.	6	48	0.3997	1.007	--	--	--	--
7.5 c.c.	--	--	--	--	44	348	0.5670	1.428
5.0 c.c.	44	351	0.7170	1.806	6	45	0.6228	1.569

terial inside the egg was more or less amorphous or globular in character with large clear hyaline-like polar bodies. In some eggs there were two polar bodies while in others only one such structure could be demonstrated. Occasionally the remains of an embryo could be identified. Upon investigation of the literature and with the assistance of Doctor Asa C. Chandler of the Rice Institute, Houston, Texas, these eggs were identified as those of *Heterodera radiculicola*. Several drawings of these eggs as they were seen in microscopic slide preparations are shown in Fig. 1.

The 39 cases discovered in this series were distributed in 16 counties in Mississippi, 10 cases coming from 8 counties in northern Mississippi and 29 from 8 counties in southern Mississippi. The majority of the cases in southern Mississippi were from 2 counties. Twenty-eight of the 39 cases were found among children fourteen years of age or less. These eggs were found both by the Stoll dilution egg-counting technic and the brine flotation method of examination. In the specimens examined by the dilution egg-counting technic,

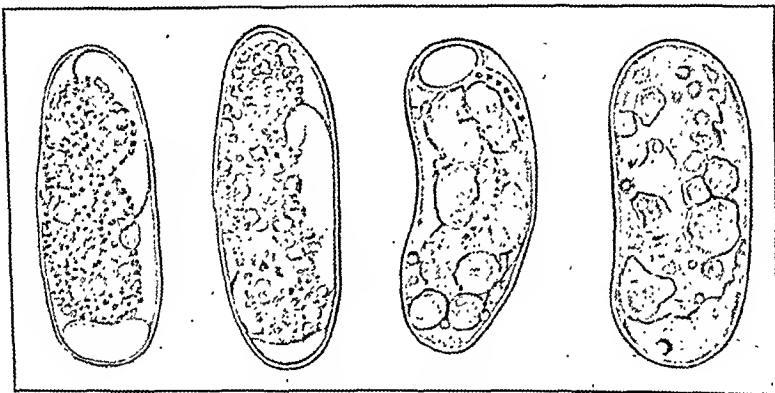


Fig. 1.—Eggs of *Heterodera radiculicola*.

there was an average of 700 eggs per gram of feces, indicating a light intensity of infestation. In the specimens found positive by the brine flotation technic, only a few eggs were found in most cases in that portion of the specimen examined microscopically.

The finding of the eggs of *Heterodera radiculicola* in human feces represents, according to Chandler, the accidental ingestion of these eggs with raw vegetables, the roots of which have become contaminated with this free living nematode. There is also the possibility that in the collection of specimens of human feces which have been deposited on the surface of the soil and examined for human helminth eggs the eggs of free living nematodes may be included from the soil contamination. This is unlikely due to the fact that adult *Heterodera radiculicola* are usually found clinging to roots and vegetables which lie deeper in the soil and not on the surface, making it unlikely that these eggs would be included in specimens of human feces obtained after coming in contact with the soil. This possibility does emphasize, however, that in the collection of specimens of feces care should be taken to prevent the inclusion of soil with the specimen.

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## THE OCCURRENCE OF EGGS OF *HETERODERA RADICICOLA* IN HUMAN FECES\*

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ALVIN E. KELLER, M.D., NASHVILLE, TENN.

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IN 1919 during the course of a hookworm study among troops at Camp Travis, Texas, Kofoed and White demonstrated nematode eggs of unusual size which were found in 429 cases from approximately 140,000 examinations of feces from soldiers. These eggs had an average dimension of 95 by 40.2 micra but varied markedly in size, the variation in length being from 68 to 133 micra and in width from 33 to 44 micra. They tentatively assigned the name of *Oxyuria incognita* to this egg, and reported their findings in the *Journal of the American Medical Association*.

In 1923 Sandground reported that the egg which was found by Kofoed and White was probably that of *Heterodera radiculicola*, a common nonpathogenic nematode which occurs widely in a large number of root vegetables, such as radishes, celery, turnips, and potatoes.

Sandground was able to show that when noninfested individuals ingested parasitized roots of beans along with the usual food it was possible to recover numerous *Heterodera* eggs the following day. Sandground does not state in his experimental observations whether worms or only eggs were swallowed with parasitized roots but he infers that the eggs rather than adult worms are ingested with food. These eggs, after they were passed from the individuals, were brown in color and were in all stages of development. They were usually symmetrically ellipsoidal but were frequently kidney shaped. They contained small fat globules. Sandground also stated that in the embryo the esophagus and esophageal bulb were similar to the same structures seen in the larvae of *Heterodera radiculicola*. Since that time the eggs which were identified by Kofoed and White have been considered to be those of *Heterodera radiculicola*.

In the course of a hookworm investigation conducted in Mississippi, similar eggs were found in the specimens of feces examined from the white and negro population of that state. These eggs were demonstrated in 34 specimens from 44,380 white persons and in 5 specimens from 6,441 negroes. The eggs varied in length from 84 to 96 micra and in width from 30 to 42 micra. They were ellipsoidal in shape but usually had a concavity on one side. Most of the eggs seen had a thin double contour. They were light brown in color and the ma-

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\*From the Department of Preventive Medicine and Public Health, Vanderbilt University. Received for publication, May 31, 1934.

# THE VIRUS OF LYMPHOGRANULOMA INGUINALE\*

## ITS CULTIVATION, ITS ANTIGENIC VALUE AS A VACCINE AND ALSO IN THE PRODUCTION OF AN ANTISERUM

JOSEPH T. TAMURA, M.Sc., CINCINNATI, OHIO

A REVIEW of the literature reveals that all attempts to cultivate the etiologic agent of lymphogranuloma inguinale have been failures. We have recently<sup>1</sup> succeeded in doing so by utilizing the medium devised by Maitland, Laing, and Lyth<sup>2</sup> for the cultivation of *Vaccinia* virus. This medium is made by placing sterile bits of rabbit tissue, kidney, liver, or spleen, in Berkefeld-filtered Tyrode's solution. We have used guinea pig tissue in place of rabbit.

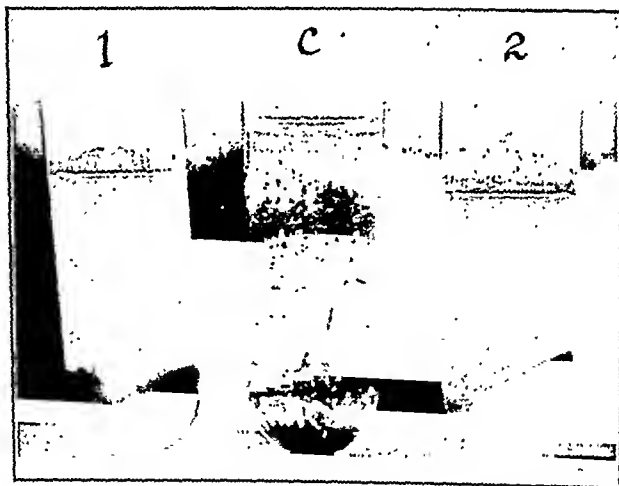


Fig. 1.—Cultivation of lymphogranuloma inguinale virus. 1, fourteenth subculture from Case C.R. C, control. 2, twelfth subculture from Case H.S., five days' incubation.

The inoculum, pus, is obtained from a fluctuant inguinal node that has not been exposed to external contamination. This pus is found to be bacteriologically sterile. It is diluted 1:5 with sterile saline solution. When from 0.02 to 0.03 c.c. of the diluted pus is planted in 7 c.c. of Tyrode's solution, containing a piece of guinea pig kidney or liver and incubated at 37.5° C., aerobically a peculiar cloudiness appears throughout the supernatant fluid in thirty-six to forty-eight hours. Control tubes of the medium, incubated in the same manner, remain perfectly clear. This cloudiness is transmissible from one subculture to the next. Fig. 1 illustrates this peculiar formation of cloudiness in the media. In our work the cloudiness was carried through 24, 14, and 12 subcultures when the procedures were discontinued.

\*From the Department of Bacteriology and Hygiene, University of Cincinnati College of Medicine, and the Cincinnati General Hospital.  
Received for publication, June 2, 1931.

Recent investigations in Ireland concerning the relationship of this parasite to soil indicate that it is most frequently found in areas where the soil is sandy. The distribution of the cases in the present study also shows this relationship to sandy soil, as a majority of the specimens were from southern Mississippi, especially in the counties where the percentage of sand in the soil varies from 56 to 88 per cent.

The presence of eggs of *Heterodera radiculicola* in the feces causes difficulty in diagnosis and may result in an error due to the fact that microscopists may not be familiar with the appearance of this nematode egg and may report the presence of some of the common eggs such as hookworm or unfertilized ascaris eggs which resemble eggs of *Heterodera radiculicola* in some respects. The failure to recognize the eggs of *Heterodera radiculicola* may not only result in an error in diagnosis but also may lead to the unnecessary administration of anthelmintics.

#### SUMMARY

1. During the course of a hookworm investigation in Mississippi the eggs of *Heterodera radiculicola* were found in 34 specimens of feces out of 44,380 specimens examined from white persons and in five instances out of 6,441 specimens obtained from negroes.

2. These eggs were found in specimens of feces examined by both the Stoll dilution egg-counting technique and the brine flotation method.

3. Only a few eggs were found in that portion of the specimen examined.

4. Of the 39 cases 28 were found among children under fourteen years of age. Twenty-nine cases were from southern Mississippi and ten were from northern Mississippi.

5. The finding of the eggs of *Heterodera radiculicola* in human feces represents the accidental ingestion of these eggs with raw vegetables, the roots of which have become contaminated with this free living nematode.

6. The presence of eggs of *Heterodera radiculicola* may result in an error in diagnosis due to their similarity in some respects to unfertilized ascaris and hookworm eggs and result in the unnecessary administration of anthelmintics.

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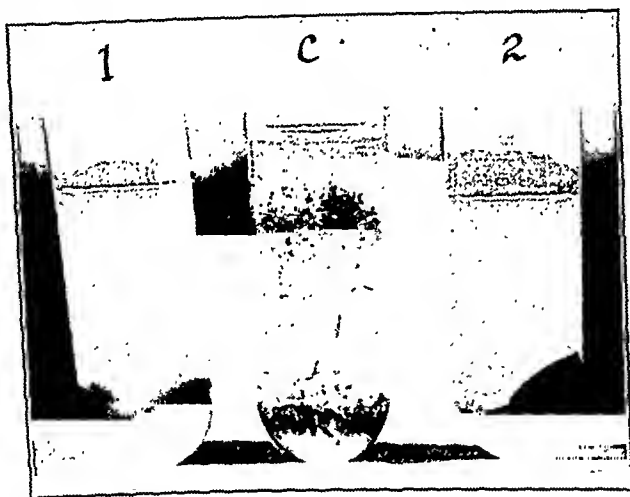


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#### SUMMARY

1. During the course of a hookworm investigation in Mississippi the eggs of *Heterodera radiculicola* were found in 34 specimens of feces out of 41,380 specimens examined from white persons and in five instances out of 6,441 specimens obtained from negroes.

2. These eggs were found in specimens of feces examined by both the Stoll dilution egg-counting technique and the brine flotation method.

3. Only a few eggs were found in that portion of the specimen examined.

4. Of the 39 cases 28 were found among children under fourteen years of age. Twenty-nine cases were from southern Mississippi and ten were from northern Mississippi.

5. The finding of the eggs of *Heterodera radiculicola* in human feces represents the accidental ingestion of these eggs with raw vegetables, the roots of which have become contaminated with this free living nematode.

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Eosin methylene blue	2.0 gm.
Azure 1	0.4 gm.
Crystal violet	0.025 gm.
Dissolve in 250 c.c. of equal parts of methyl alcohol and glycerin; filter through paper.	

One c.c. of the stock solution is diluted in 50 c.c. of distilled water just before using. Fixed and eosin-treated slides are stained from fifty to seventy minutes at room temperature, are washed in water and dried in the air.

It is found that not all of the bubonic pus from the patients produce cloudiness of the medium. When pus from patients that fails to produce cloudiness in media is used to prepare Frei antigen, this antigen fails to give positive skin reactions in proved cases. Subcultures from a 1:10,000 dilution of pus failed to produce cloudiness, but from a 1:1,000 dilution yielded growth. When diluted pus or the cloudy supernatant fluid in culture is passed through a

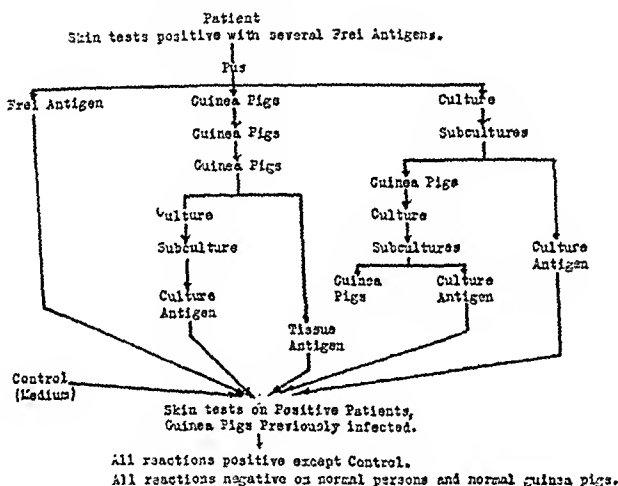


Chart 1.

Berkefeld "N" filter the filtrate will produce cloudiness on subculture. When 1 c.c. of the cloudy supernatant fluid is inoculated into the groin of a guinea pig, a lymphadenitis is produced in from two to three days. The glands gradually increase in diameter and after ten to twelve days will become conglomerate masses of glands and may measure 15 mm. or more in diameter. The overlying skin becomes adherent to the affected nodes, after which they tend to disappear spontaneously. The skin sensitivity of the animals will develop in from three to four weeks. This is comparable to that which one can accomplish by direct inoculation with human pus. Such culture inoculations were successful when the fourth, sixth, and eighth subcultures were used. The etiologic agent has been passed from culture to guinea pig, then from guinea pig to the medium, from this culture to a subculture and then back to the guinea pig. Chart 1 illustrates the complete cycle of animal inoculation.

The cloudy supernatant fluid from a subculture was heated to 60° C. for two hours on one day, and at the same temperature for one hour on the fol-

In our experience, as in that of others, cultures made from bubonic pus when planted on a wide variety of bacteriologic media and grown aerobically, at partial oxygen tension, or anaerobically, remain free of visible growth. The cloudiness in the tissue-Tyrodé medium remains for twelve- to fourteen-day incubation periods, after which the cloudiness seems to precipitate out and the supernatant fluid becomes clear and slightly straw colored. When this clear supernatant fluid, which has been cloudy once, is subcultured, it no longer produces cloudiness.

When the cloudy supernatant fluid is examined or cultured nothing resembling ordinary bacteria is to be found. Attempts to see the etiologic agent in cloudy fluid have failed with the exception that peculiar granules are brought out by Giemsa's stain and by eosin-Giemsa method originated by Hosokawa<sup>3</sup> for the staining of a variety of viruses. Hosokawa's method of staining viruses, which we used, is as follows:

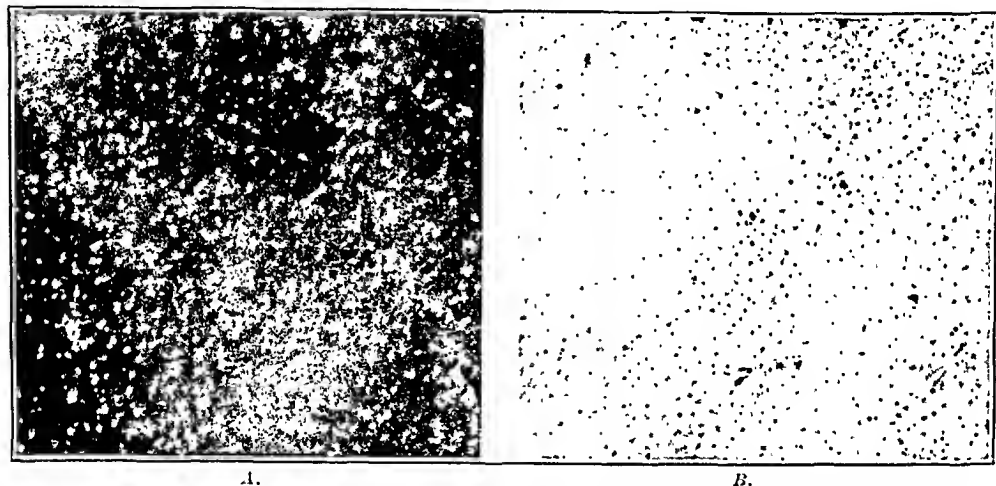


Fig. 2.—Cloudy supernatant culture fluid stained with Hosokawa's method, mounted in hyrax. A, Dark-field, reduced from a photomicrograph of 1500 diameters. B, Direct illumination, reduced from a photomicrograph of 1100 diameters.

1. *Smearing*: Loopful cloudy supernatant fluid is smeared as thin as possible on a perfectly clean slide by means of drawing it lengthwise by a second slide, as in blood smear preparation. The smear is air dried.

2. *Fixation*: The smeared film, after thorough drying in air, is fixed for two minutes in the fixing solution, then washed in water.

The fixing solution:

Methyl alcohol	100.0 c.c.
Formalin (original solution)	5.0 c.c.
Glacial acetic acid	1.0 c.c.

3. *Treating with eosin*: Flood the fixed slide with 1 per cent eosin solution and heat it over a flame for from one-half to one minute until it just steams, as in the case of staining acid-fast bacilli. Wash with water carefully.

4. *Giemsa staining*: Three drops of stock Giemsa in 2 c.c. distilled water is used. Hosokawa solution gave better results. This solution is prepared as follows:

CASE 2.—T. D., white male, single, twenty-seven years of age. He was exposed to infection Dec. 17, 1933. Onset of swelling in left groin was first noticed Jan. 2, 1934. Admitted to hospital January 19, and seen on January 31. There was tenderness on palpation of the affected glands, which measured 6 by 3 cm. The skin was attached to the

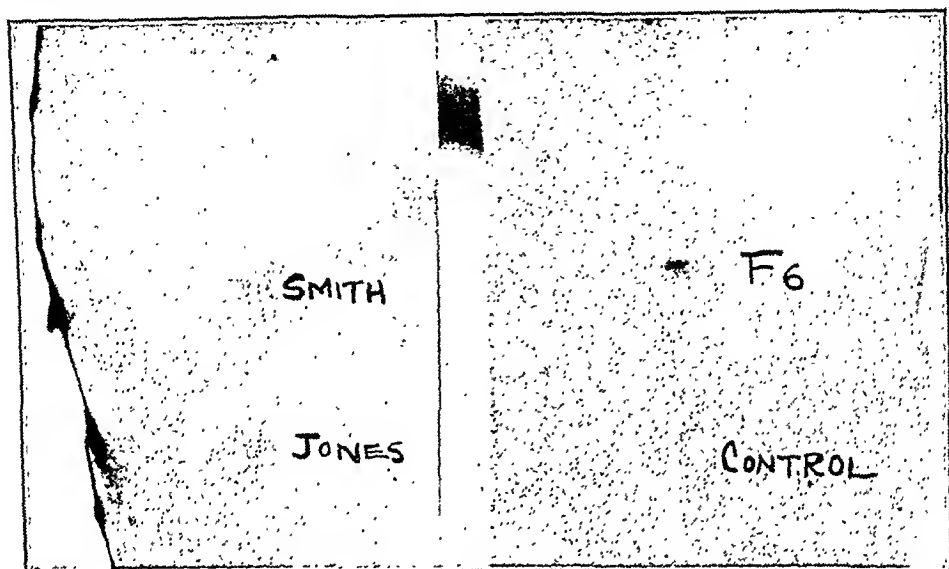


Fig. 3.—Frei skin tests, Case T.D. *Smith*, Antigen prepared from Smith. *Jones*, Antigen prepared from Jones. *F<sub>6</sub>*, Culture antigen (sixth subculture). *Control*, Tissue Tyrode medium.

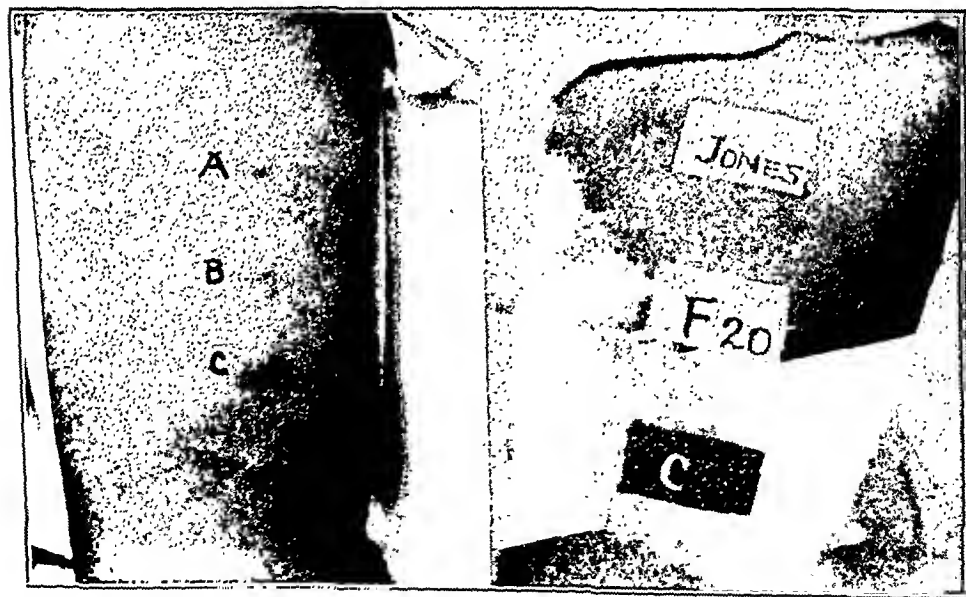


Fig. 4.—Left, Case R.S. *A*, Frei antigen. *B*, Frei antigen. *C*, Culture antigen (sixth subculture). *D*, Control with tissue Tyrode medium. Right, Case E.T. Jones, Frei antigen. *F<sub>20</sub>*, Culture antigen (twentieth subculture). *C*, Control, tissue Tyrode medium.

underlying nodes, dark reddish purple in color. No masses in right groin. No history of syphilis, and Kahn test negative. Frei skin tests markedly positive. The nodes suppurred and pus was aspirated on February 14, 3 c.c., and again 2 c.c. on February 20. Bacteriologic examinations of the pus, made twice, were entirely negative. Beginning Feb-

lowing day, as in the well-known method of preparing Frei's antigen from pus. When such heated culture antigen, preserved with 1:10,000 merthiolate, is injected intradermally into patients it gives just as marked reactions as does the Frei antigen. When the twenty-third subculture in one series was heated for antigen it was found to be just as active as antigen prepared from earlier subcultures. As in the case with Frei's antigen, the culture antigen gives no reaction in normal individuals.

An attempt has been made to produce lymphogranuloma inguinale antiserum from a goat with heated culture antigen. Beginning on Dec. 1, 1933, and continuing to March 28, 1934, a total of 81 c.c. of the antigen was injected into the animal subcutaneously. The animal was bled on April 6, and 145 c.c. of serum was obtained. Merthiolate, in 1:10,000 concentration was added as preservative. Gottlieb<sup>4</sup> reported experimental evidence to show that there are in the serums of patients recovering from lymphogranuloma inguinale substances which have neutralizing properties on the Frei antigen when this is used for skin tests. In our work intradermal injections were made into lymphogranuloma inguinale patients of (1) Frei antigen, (2) saline, (3) Frei antigen plus normal goat serum, (4) Frei antigen plus antilymphogranuloma inguinale goat serum, after the mixture had been kept under refrigeration for forty-eight hours. (1) and (3) gave positive reactions while (2) and (4) were negative. This antiserum from the goat has been used for the treatment of two patients, reported below.

Through the courtesy of the Department of Dermatology of the Cincinnati General Hospital we are able to present thirteen cases we have treated using heated culture antigen as a vaccine administered subcutaneously, and two cases treated with antiserum. Eleven cases, including one serum-treated case, showed such marked improvement that they were considered cured in an average of eight weeks. Usually the vaccine is given with an initial dose of 0.2 to 0.3 c.c., which gives strong local reaction. Each subsequent injection produces a smaller and smaller response. The tolerance to larger doses given later is believed to be due to desensitization. Accompanying the clinical improvement there was a marked reduction in skin sensitivity. Of the remaining four cases, three are chronic and one has been treated for only two weeks. These appear to be following a similar favorable course. Beneficial therapeutic effects following the intradermal inoculation of Frei's antigen have been reported recently by Wien and Perlstein<sup>5</sup> who refer to the previous work of Herman and Gay-Prieto. Brief accounts of several cases follow. Treatment of four cases with heated culture antigen.

CASE 1.—E. T., negro male, single, twenty years of age. He was first seen on Dec. 14, 1933. There were two chains of swollen glands in the right groin, measuring 5 by 2 cm., and 7 by 2.5 cm. There were no palpable masses in the left groin. Patient first noticed the "swelling of groin" in latter part of September, 1933, and had been under treatment since October 4 by various physicians. There was no history of syphilis and Wassermann reaction was negative. Gonococcus infection one year ago. Frei skin tests strongly positive. Beginning December 18, with initial dose of 0.1 c.c., a total of 22.7 c.c. of vaccine was given, in 30 inoculations. Considerable reduction in skin sensitivity was obtained. On March 8, when the last inoculation was given, both chains of nodal masses had disappeared completely. No remission has occurred in two months.

TABLE I\*  
GIVING DATA ON TEN CASES NOT REFERRED TO IN TEXT

CASES	AGE, SEX, COLOR	DATE OF ONSET	INCUBATION PERIOD (DAY)	DURATION (WEEK)	PRIMARY LESION	INGUINAL ADENITIS	SUPPURATION	FISTULATION	LENGTH OF TREATMENT (WEEK)	NUMBER OF INJECTIONS	TOTAL VACCINE GIVEN (C.C.)	WASSERMANN OR KAHN REACTIONS	RESULTS
R.S.	17 M. B.	12/25/33	10	4	+	U	+	+	10	27	28.4	-	C
A.R.	19 M. B.	1/19/34	14	3	+	U	+	+	9	26	27.0	-	C
H.K.	19 M. B.	2/ 8/34	7	8	-	B	-	-	5	11	8.3	-	C
C.F.	28 M. W.	3/15/34	10	2	-	U	+	+	7	21	16.0	-	C
R.M.	31 M. B.	2/19/34	9	6	-	B	-	-	6	17	10.0	-	C
S.B.	35 M. B.	3/ 9/34	?	3	+	U	+	L	8	16	11.5	-	C
B.R.	18 M. B.	July, '33	?	24	?	B	+	+	17	40	32.0	-	G. I.
H.S.	46 M. B.	Jan., '34	?	10	?	U	+	+	8	21	16.0	?	I
G.S.	26 M. B.	4/22/34	14	2	+	U	+	L	2	3	Anti-Serum 60.0	?	G. I.
J.W.	39 F. B.	July, '33	?	36	?	U	+	+	9	27	16.3	-	G. I.

\*Keys to the Table: U, Unilateral.  
C, Cured.  
L, Lanced.

B, Bilateral.  
I, Improved.  
1 and G. I., still under treatment.  
G. I., Greatly Improved.

CASE 3.—C. S. J., negro male, single, thirty-one years of age. He had had inguinal adenitis for about two weeks when he was first seen, on December 28. Markedly swollen, fluctuant nodes in the right groin. No masses in the left groin. He had had gonorrhea four years ago and again four months ago. No lesion on or discharge from the penis. Kahn reaction 2-plus. Frei tests markedly positive. He was admitted to the hospital December 28. On the next day 5 c.c. of pus were aspirated from the fluctuant nodes. Dark-field examination for treponemas was negative; cultures negative. The suppurating nodes ruptured spontaneously January 2. Patient discharged from hospital January 12 and readmitted January 29. The nodes were very swollen and tender and there was a small fistula from which purulent material was discharging. Vaccine therapy was begun January 29, with initial dose of 0.3 c.c. A total of 23.5 c.c. of vaccine was injected in 28 inoculations. Patient resumed his occupation as a porter on March 17, and when last seen on April 9 the fistula was healed and masses of nodes were no longer palpable.

CASE 4.—F. M., negro male, married, thirty years of age. He was seen January 11. He had bilateral inguinal swellings. Indurated masses of nodes measured 6 by 2 cm. on left, and 7 by 2.5 cm. on right. The swelling was first noticed by the patient on December 25.

ruary 1 and continuing to April 5, 25 subcutaneous injections of vaccine were given, totaling 22 c.c. Skin sensitivity was reduced to a negligible degree. When last seen, on April 18, there were no palpable masses.

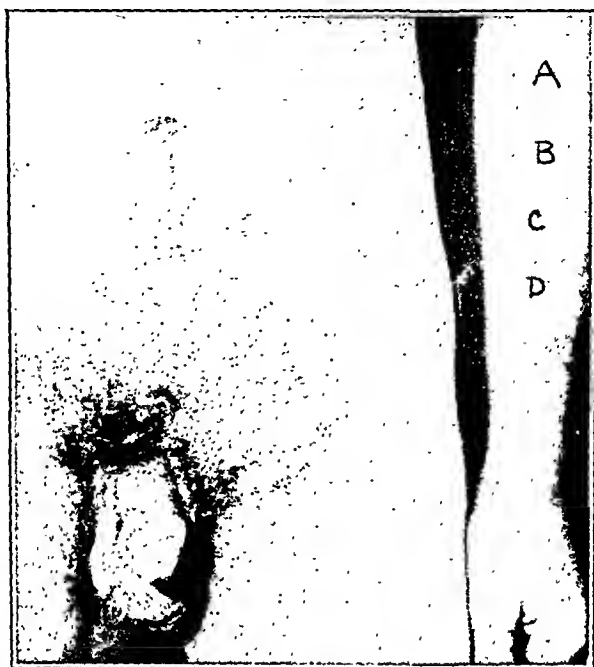


Fig. 5.—Unilateral lymphogranuloma inguinale. Positive Frei reactions on arm. *A*, Frei antigen. *B*, Culture antigen (fifteenth subculture). *C*, Frei antigen. *D*, Control with tissue Tyrode medium.



Fig. 6.—Unilateral lymphogranuloma inguinale; Case R.S., aged seventeen. Arrow points to characteristic primary lesion.



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## WATER STUDIES IN OBESITY\*

ELMER C. BARTELS, M.D., SPRINGFIELD, ILL., AND BENJAMIN BLUM, M.D.,  
ROCHESTER, MINN.

REFERENCES have been made in the literature to disturbed water metabolism in obesity. Bauer mentioned the frequent association of obesity and disturbance of the water and salt balance. Grafe noted the tendency of obese patients toward water retention. Clasen suggested that this disturbance of water balance was due to the influence of the glands of internal secretion.

The question of water balance arises whenever reduction of weight of obese patients is attempted. Newburgh and Johnston, and Newburgh and Lashmet have reported the tendency of obese patients who are on a low calorie diet to retain water which prevented loss of weight and in some cases caused a gain of weight for some days. However, in the long run the body weight almost always approached the predicted figure as an accurate tabulation of the water exchange indicates that body weight is the result of two factors, namely: (1) gain or loss of tissue, and (2) gain or loss of water. Grote and Calo advised the study of water metabolism as a guide in the selection of therapeutic methods of reduction. Evans and Strang felt that this water retention during attempts at reduction is due to the spontaneous alteration in the capacity of fat to store water. So prominent is this matter of water retention that diuretics have been considered and used by Grafe and Zolotareva and Kogan as an adjunct to a low calorie diet. In twenty-eight of thirty-one cases Zolotareva and Kogan, by the use of 1 to 2 c.c. of mersalyl, obtained a considerable increase in the amount of urine. This sometimes reached 3 liters a day. None of these patients was suffering from cardiac disturbances. Grafe restricted the fluid intake and counteracted this tendency to water retention by the use of merbaphen.

### TESTS OF DILUTION AND CONCENTRATION

As an approach to this fundamental problem we reviewed the work on dilution and concentration tests in diseases other than those of the kidney. Pratt was the first to study this subject in this country. From studies on control subjects, he concluded that in normal individuals the output of urine in four hours should be equal to or greater than the intake at the onset. No series of obese cases was considered. Buck and Proger conducted similar experiments.

\*Read before the Annual Meeting of the Staff of St. Luke's Hospital, Duluth, Minnesota, January 13, 1934.

Received for publication, June 9, 1934.

Ten days preceding the onset he was exposed to infection. There was no sore on the penis. Gonorrheal infection two years previously. Kahn reaction negative. Frei skin tests strongly positive. The nodes never supplicated. Beginning January 13, a total of 19 c.c. of vaccine was given in 22 subcutaneous injections. Marked desensitization was obtained. The masses of nodes had completely disappeared from both groins by March 5. He resumed his occupation as a porter on this date, and has had no remission for three months.

### Treatment of one case with goat antiserum.

CASE 5.—C. R., white male, single, 25 years of age. He was seen first February 8, with markedly swollen nodes in left groin, measuring 10 by 5 cm., very tender and painful. Swelling of groin was first noted January 26, a week or ten days after he was exposed to infection. No primary lesion found. Slight urethral discharge, which upon examination was found to be gonorrheal. Kahn reaction 3-plus. Frei skin tests strongly positive. The involved nodes supplicated and pus aspirated: February 17, 3 c.c.; February 19, 3 c.c.; February 24, 6 c.c.; February 26, 6 c.c., and March 1, 8 c.c. Bacteriologic examination of the pus negative. Patient was admitted to hospital March 1. Beginning February 10, 9.4 c.c. of vaccine was given subcutaneously in 13 inoculations. On March 8, the nodes were incised and curetted, when vaccine therapy was discontinued. Seropurulent discharge continued and wound did not heal. Swelling of suprapubic nodes noted April 7. Patient was dismissed from hospital on April 10, readmitted on April 18, when the suprapubic nodes were incised and curetted. Continuous drainage followed. Skin test with a goat serum was negative on April 23; followed by 20 c.c. of antiserum intravenously. Twenty cubic centimeters of antiserum was given again on the twenty-fourth, and 15 c.c. by vein on the twenty-sixth. Serum sickness occurred on the twenty-ninth and lasted thirty-six hours. Patient was discharged from hospital May 4. Drainage had ceased and epithelialization had occurred. Two weeks after the first serum injection the wound was completely healed.

### SUMMARY

When pus from the lymphogranuloma inguinale is planted in the medium devised by Maitland and his coworkers for the cultivation of *Vaccinia* virus, the medium becomes cloudy. The etiologic agent produces a cloudiness which is transmissible in serial cultures, or serial cultures alternating with guinea pig inoculations. The etiologic agent in pus or in cloudy supernatant culture fluid passes the Berkefeld "N" filter. The virus is stainable by Hosokawa's cosin-Giemsa method. The heated cultures have been used successfully in making diagnosis by the intradermal skin test, and in inducing recoveries by subcutaneous inoculations. Also heated cultures have been used to produce an antiserum. Although one can draw no definite conclusions from the few cases treated with antiserum, the results in these cases justify further trial of serum therapy.

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TABLE I

STUDIES OF DAILY WEIGHT, WATER INTAKE AND OUTPUT OF A PATIENT ON A DIET OF 709 CALORIES

DATE	DAYS ON DIET	DAILY CHANGE IN WEIGHT POUNDS	FLUID INTAKE C.C.	FLUID OUTPUT C.C.
July 17	1	265	600	875
July 18	2	265 to 262½	1,200	2,500
July 19	3	262½ to 260	1,275	850
July 20	4	260 to 259	1,830	1,500
July 21	5	259 to 258	1,200	1,350
July 22	6	258 to 257	1,175	775
July 23*	7	257 to 253	1,275	2,450
July 24	8	253	1,200	650
July 25	9	253 to 254½	1,000	1,000
July 26	10	254½	1,200	600
July 27	11	254½ to 253½	1,200	1,400
July 28*	12	253½ to 249½	1,200	3,200
July 29	13	249½ to 250	1,510	1,300
July 30	14	250 to 249	1,160	950
July 31	15	249		
August 1*	16	249 to 245	1,200	4,000
August 2	17	245	1,165	1,600
August 3	18	245	2,700	3,100
August 4	19	245		
Total			22,090	28,100†

\*2 c.c. of salyrgan intravenously.

†Difference between total intake and output of fluid is equal to 13 pounds.

started, because of the low carbohydrate intake of approximately 60 gm. we were able to discontinue the insulin completely without subsequent sugar in the urine and with a normal blood sugar. On the seventh day edema of the ankles was first noticed. As such edema seemed to need diuretic therapy, the patient was given 2 c.c. of salyrgan intravenously. There was a subsequent diuresis of 2,450 c.c. on an intake of 1,275 c.c. and a loss of weight of 4 pounds. The edema was apparently relieved. The edema recurred on the twelfth day so 2 c.c. of salyrgan was given again. This was followed by diuresis of 3,200 c.c. on an intake of water 1,200 c.c. Weight decreased 4 pounds. After another four days this procedure was repeated with an output of urine of 4,000 c.c. on an intake of 1,200 c.c. of water. An additional loss of weight of 4 pounds occurred. The output of 1,600 of urine on the seventeenth day after the diet was begun was thought to be latent response to the salyrgan. The output of 3,100 c.c. of urine on the eighteenth day in spite of intake of 2,700 c.c. of water could not be explained at that time; however, we were able to make a plausible explanation later.

The total intake of fluid for the nineteen days in hospital was 22,090 c.c. and the total output was 28,100 c.c. The output exceeded the intake by 6,010 c.c. (13 pounds). If this is added to the actual weight (245 pounds) a total of 258 pounds is obtained which is approximately 2 pounds more than the calculated weight of 256 pounds. This indicates that the forced weight loss was entirely at the expense of the body fluids and explains the reason for the ultimate outcome in which the actual loss and the calculated loss of weight are almost identical at the end of ten weeks (Table I).

In one month, after the patient had lost consistently a total of 30 pounds, another dilution test was carried out with the following results: The output of urine for the first hour was 535 c.c. with a specific gravity of 1.005; for the second hour 1,135 c.c. with a specific gravity of 1.004; for the third hour, 160 c.c. with specific gravity of 1.006; and for the fourth hour 80 c.c. with a specific gravity of 1.009. The total output for the four hours was 1,910 c.c.

This excessive response, we felt, was due entirely to the diuretic action of water. The low caloric diet had caused a disturbance in the water balance and the large intake of water

The average output of urine in four hours after the ingestion of 1 liter of water was 1,122 c.c. In fifteen cases the output exceeded 1,500 c.c. in four hours. Of these, there were twelve cases of hypertension, one of neurasthenia, one of cardiosclerosis, and one of secondary anemia.

Janney and Walker, testing water balance in pregnancy, found that normal control subjects who had ingested 1,200 c.c. of water eliminated 1,353 c.c. in four hours, whereas during pregnancy the output declined progressively among clinically normal, pregnant women in the last twelve weeks of pregnancy. He attributed this to a lower functional capacity of the kidney in the latter weeks of pregnancy and considered this as a precipitating factor in toxemic states.

Malmud was the first to make studies on the disturbance in the dilution tests of Volhard in obesity. She carried out the test in various types of obesity, giving 1,000 c.c. of warm tea flavored with sugar and collecting the urine for the next four hours. She found disturbances in the output of urine which were in proportion to the severity of the obesity. The disturbance in water balance was manifested by reduction in the output of urine during four hours. A critical study of her report leaves one in doubt as to the validity of her conclusions.

Our study was precipitated by the following case which was studied in the hospital during the management of weight reduction.

#### REPORT OF CASE

The patient was a female, forty-four years of age, who was 65 inches tall and weighed 265 pounds. The present weight had been maintained for several years. She had had a severe type of diabetes for many years which was completely controlled by diet and 90 units of insulin a day. We felt that if the weight could be reduced a corresponding improvement in the diabetes would take place.

Physical examination, except for the marked obesity, was negative. There was no edema of the extremities. The blood pressure was and had always been within normal limits. The laboratory tests which included urine, blood counts, and blood chemistry gave normal results. Before beginning a dietary regimen a dilution test was done, which consisted in giving the patient 1,500 c.c. of water at 8:00 A.M. after a relatively dry breakfast. The urine was collected hourly for the next four hours. The following results were obtained: At the end of the first hour 170 c.c. of urine with a specific gravity of 1.009 had been excreted; during the second hour 280 c.c. with a specific gravity of 1.006; during the third hour 140 c.c. with a specific gravity of 1.007, and during the fourth hour 115 c.c. with a specific gravity of 1.008. A total of 705 c.c. of urine was excreted in the four hours.

This total was markedly below the reported normal in which intake equals output. As the urinalysis and tests of blood chemistry had given normal results, and there was apparently no edema or dehydration, we felt that we were justified in explaining this result as a disturbed water balance. Some criticism may be made on the fact that we performed only one such test before starting the patient on the reduction diet. The part the associated diabetes played in the output of urine can only be suggested.

The patient, while being up and around the entire day, was placed on a diet of 709 calories. Daily studies were made of weight, water intake and output. On the second day of the diet the patient had a marked spontaneous diuresis with a corresponding loss of weight of 2½ pounds (Table I). This sudden loss of weight can be attributed to the sudden stopping of the large dose of insulin, as described by MacBryde. For on the day the diet was

were more than 10 per cent overweight and those more than 10 per cent underweight were compared with the normal controls. The percentage of excretions of more than 1,500 c.c. and less than 1,000 c.c. was determined for the different groups.

## RESULTS

From the tabulations in Tables II, III, and IV, it is at once seen that there is a wide variation in the hourly and total outputs in the four groups. In the obese group with the three and four determinations for Subjects 109, 110, 111,

TABLE II  
RESULTS OF DILUTION TESTS ON SUBJECTS WHO WERE UNDERWEIGHT

UNDERWEIGHT POUNDS	CASE NUMBER	FIRST HOUR OUTPUT C.C.	SECOND HOUR OUTPUT C.C.	THIRD HOUR OUTPUT C.C.	FOURTH HOUR OUTPUT C.C.	TOTAL OUTPUT C.C.
<i>Seven Subjects More Than 10 Per Cent Underweight</i>						
19.0	7	500	710	335	90	935
17.0	46	490	445	230	35	1,200
14.0	5	380	700	52	35	1,167
13.0	65	300	430	430	84	1,244
12.0	40	135	200	540	75	950
10.0	18	435	555	230	60	1,280
10.0	4	225	450	100	20	795
<i>Twenty-Two Subjects 0 to 10 Per Cent Underweight</i>						
12.0	74	163	540	104	40	852
12.0	6	136	224	212	35	607
9.0	49	235	730	455	40	1,460
8.0	104	800	550	290	20	1,660
8.0	21	320	615	330	55	1,320
7.0	51	300	745	200	30	1,275
7.0	37	590	550	425	100	1,665
6.0	78	310	170	120	105	705
5.0	53	480	770	460	35	1,745
5.0	42	60	460	430	135	1,085
4.0	69	330	450	520	122	1,422
4.0	61	185	380	370	70	1,005
4.0	10	240	485	480	355	1,560
3.5	101	925	250	90	250	1,515
3.5	86	200	475	550	125	1,350
3.0	95	500	475	475	100	1,550
2.5	68	380	840	370	26	1,616
2.0	76	90	400	425	85	1,000
1.0	19	400	540	75	20	1,035
1.0	8	185	610	100	30	925
0.5	29	455	245	125	32	857
0.5		740	550	132	32	1,454

112, and 113, this range is also seen. As this variation is present in all the groups we felt that if any comparison or difference was noted, it would only be secured by averaging the groups.

Table V is a complete tabulation of the average hourly output and average total output in each of the groups. We concluded that a normal output, if there is such an entity, could best be obtained by averaging the output of the groups from 0 to 10 per cent overweight and from 0 to 10 per cent underweight. The average total output of urine is then calculated to be 1,334 c.c. in four hours

was sufficient to start diuresis. This suggested to us the apparent benefit of taking large quantities of water during obesity to force loss of weight for psychic reasons instead of a reduction in fluid intake as advised by some authors. However, this rapid forced loss of weight by the use of diuretics did not materially affect the ultimate loss as is shown by Fig. 1. This patient has continued on the diet and the loss of weight has been constant and follows closely the estimated loss.

### METHOD

We were forced from this case to accept the presence of a disturbed water balance in obese patients during efforts at reduction by the use of a low caloric diet. Our problem was to determine the water balance in obese persons not on a reduction diet and to see if it was at all disturbed as has been suggested. To do this we made determinations on the members of the nursing staff of two large Duluth hospitals and added to this a group of obese persons which included private and dispensary patients. From this number we were able to obtain a large list of normal control subjects as well as subjects who were

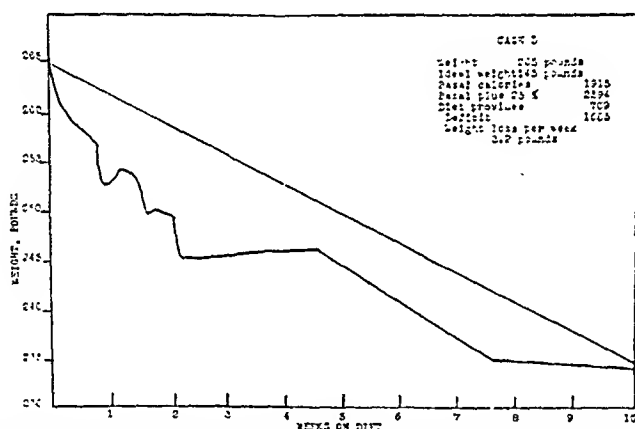


Fig. 1.—Upper line represents the estimated weight; the lower line is the actual weight. The black dot at 258 pounds at about three weeks on diet is the actual weight plus the weight of the excess water which had been lost.

under- and overweight. The entire group consisted of 102 subjects on whom 117 determinations were made. The test consisted of giving 1,500 c.c. of water at 8:00 A.M. and collecting the urine at hourly intervals for the next four hours during which time the subjects were up and around carrying on their usual duties. The amounts collected each hour, their specific gravity, and the total output were ascertained in each case. A routine urinalysis was made on the concentrated fourth hour specimen. Only those cases were included in the final consideration in which urinalysis and blood pressure were normal and physical examination was grossly negative. The subjects were normal except for the variations in weight. All were females, but we felt that this should not invalidate the results.

The age, weight, and height of each subject were taken and the normal weight was ascertained from an accepted table of normal weights. From the actual weight and the normal weight the percentage over- and underweight was calculated. We took as normal all those subjects who were within the range of 10 per cent overweight and 10 per cent underweight. Those subjects who

TABLE IV  
RESULTS OF DILUTION TESTS ON THIRTY-TWO SUBJECTS WHO WERE MORE THAN 10 PER CENT OVERWEIGHT

OVERWEIGHT POUNDS	CASE NUMBER	FIRST HOUR OUTPUT C.C.	SECOND HOUR OUTPUT C.C.	THIRD HOUR OUTPUT C.C.	FOURTH HOUR OUTPUT C.C.	TOTAL OUTPUT C.C.
10	9	275	120	70	40	505
10	35	155	165	135	75	530
10	73	485	205	130	80	900
10	100	500	650	50	100	1,300
11	22	305	550	375	45	1,275
11	24	200	530	90	30	850
12	64	630	620	144	50	1,440
12	70	810	700	450	50	2,000
12	108	1,000	850	150	25	2,025
13	44	420	480	180	55	1,135
13	82	800	825	150	20	1,795
14	103	500	600	300	50	1,450
15	20	315	395	125	30	865
15	105	900	700	150	100	1,850
16	79	285	465	260	95	1,165
16	87	600	600	500	120	1,820
17	96	500	500	500	50	1,550
18	83	900	650	300	150	2,000
20	48	555	670	255	40	1,520
20	111	95	320	235	45	695
		35	200	475	350	1,060
		55	500	575	285	1,415
		195	600	350	70	1,215
21	107	550	650	400	110	1,710
22	3	350	875	210	52	1,487
22	11	165	500	145	35	845
22	60	155	375	70	50	650
24	14	550	360	175	60	1,145
29	34	250	435	490	120	1,285
29	58	350	570	290	110	1,320
31	113	395	380	455	320	1,550
		180	375	450	560	1,565
		270	470	470	235	1,445
		265	265	450	230	1,310
38	109	210	580	460	35	1,285
		100	590	435	175	1,300
		170	725	430	75	1,400
		210	605	480	220	1,515
40	110	235	620	185	40	1,080
		175	580	355	90	1,200
		250	655	240	55	1,200
46	112	240	310	330	220	1,200
		365	410	300	200	1,275
		225	395	345	205	1,170
		270	230	250	210	1,070
75	15	170	280	140	115	705

with that of underweight subjects, is that a sufficiently wide range in the amount underweight was not available for absolute comparison with the group of overweight subjects.

Table VI is an additional determination of the percentage of subjects in the four groups which had outputs of urine of more than 1,500 c.c. or of less than 1,000 c.c. Of apparent significance is the fact that only 28 per cent of the group that was more than 10 per cent overweight had an output of over 1,500 c.c. However, the difference is not great. The 7 per cent of the group

TABLE III

RESULTS OF DILUTION TESTS ON FORTY-TWO SUBJECTS WHO WERE FROM 0 TO 10 PER CENT OVERWEIGHT

OVERWEIGHT POUNDS	CASE NUMBER	FIRST HOUR OUTPUT C.C.	SECOND HOUR OUTPUT C.C.	THIRD HOUR OUTPUT C.C.	FOURTH HOUR OUTPUT C.C.	TOTAL OUTPUT C.C.
0.0	52	210	680	370	327	1,587
0.0	55	630	455	410	55	1,550
0.0	89	650	700	300	90	1,740
1.5	93	200	850	350	75	1,375
2.0	25	195	680	460	45	1,380
2.0	56	410	550	240	30	1,230
2.0	59	340	590	110	35	1,075
2.0	90	425	725	650	120	1,920
2.0	92	500	700	200	100	1,500
2.5	1	260	425	300	120	1,105
2.5	85	250	850	425	75	1,600
3.0	36	200	750	250	35	1,235
3.0	63	210	420	185	30	845
3.0	84	625	850	200	75	1,750
3.0	88	500	750	400	120	1,775
3.0	102	650	600	150	50	1,450
4.0	28	225	665	430	325	1,645
4.0	66	370	480	240	40	1,130
4.0	30	435	455	145	55	1,090
4.0	47	740	455	435	35	1,665
4.0	99	300	750	200	75	1,325
4.5	2	250	750	210	30	1,240
5.0	26	870	640	180	35	1,725
5.0	43	260	565	350	210	1,395
6.0	54	870	475	135	75	1,555
7.0	23	360	400	430	235	1,425
7.0	38	490	425	330	300	1,545
7.0	45	330	620	245	60	1,255
8.0	39	125	550	600	45	1,320
8.0	57	240	570	415	170	1,395
8.0	72	460	660	550	70	1,740
8.0	94	600	600	250	50	1,500
8.5	75	530	455	96		1,081
8.5	106	600	825	250	100	1,775
9.0	7	130	544	210	24	908
9.0	50	205	225	55	35	520
9.0	67	600	580	470	105	1,755
9.0	98	600	500	510	100	1,710
10.0	27	410	395	297	40	1,142
10.0	62	290	450	370	90	1,200
10.0	91	500	800	450	180	1,930
13.0	31	385	460	560	85	1,490

after an intake of 1,500 c.c. of water. The average hourly output is 389 c.c., 545 c.c., 311 c.c., and 88 c.c. in the four hours, respectively. At once it is seen that both the group of subjects more than 10 per cent underweight and that more than 10 per cent overweight have total outputs below the normal of 1,334 c.c.; the former more than the latter. An apparently significant figure is the fourth hour output in the group more than 10 per cent overweight. This figure of 119 c.c. suggests the tendency of obese patients to have a disturbance which is not marked in total output but in the manner of output. A sustained output was always noticed by the relatively increased fourth hour output. An error, which may be suggested in this comparison of the group of overweight subjects



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TABLE V  
AVERAGE TOTAL OUTPUT OF URINE FROM EACH OF THE FOUR GROUPS

WEIGHT	HOUR				TOTAL, C.C.
	FIRST	SECOND	THIRD	FOURTH	
0 to 10 per cent underweight	364	502	304	83	1,255
0 to 10 per cent overweight	415	589	319	94	1,414
Average	389	545	311	88	1,334
More than 10 per cent underweight	338	498	272	57	1,179
More than 10 per cent overweight	359	490	296	119	1,269

TABLE VI  
PER CENT OF SUBJECTS IN EACH GROUP WITH OUTPUT OF MORE THAN 1,500 C.C. AND LESS THAN 1,000 C.C. OF URINE

MORE THAN 1,500 C.C.	PER CENT
0 to 10 per cent underweight	33
0 to 10 per cent overweight	40
More than 10 per cent underweight	23
More than 10 per cent overweight	34
LESS THAN 1,000 C.C.	
0 to 10 per cent underweight	23
0 to 10 per cent overweight	7
More than 10 per cent underweight	28
More than 10 per cent overweight	28

which was from 1 to 10 per cent overweight who had an output of less than 1,000 c.c. of urine is significant in that it indicates that obese subjects retain water to a much less degree than those in the other group.

#### SUMMARY AND CONCLUSIONS

Disturbance in water balance does occur during attempts at reduction in weight in cases of obesity as is shown in the literature and the case reported. In obese subjects who are not on diets, there is some slight evidence of retention of water as is indicated by the Volhard dilution test. This retention of water is more marked in underweight subjects. In obese subjects there is evidence of a sustained output, as indicated by the moderately high output of 119 c.c. as against a normal output of 88 c.c. in the fourth hour, as well as a slight reduction in total output. Obese subjects do not retain fluids to a great degree, as in only 7 per cent was the output less than 1,000 c.c. Underweight subjects have smaller total outputs in four hours than obese subjects.

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used filter paper instead of plaster of Paris as a medium upon which to dry the blood, and reported values that favorably compared with those obtained by the method of Myers. The method was further modified by Ling.<sup>6</sup> However, Allardyce<sup>7</sup> found that the use of filter paper, cotton batting, and similar media as desiccating agents gave results far below those obtained when plaster of Paris was employed. This same finding was confirmed by Bernhard and Dreker.<sup>8</sup> They found that chloroform did not extract over two-thirds of the cholesterol from serum dried on filter paper, but they did find that an equal mixture of alcohol and ether gave a complete extraction of cholesterol from serum dried on this medium, and checked their values against digitonin determinations. These workers employed a simple extracting apparatus composed chiefly of a Folin-Wu tube and test tube condenser, and carried out the colorimetric estimation in the same tube. Such an apparatus had already been employed by Harnes.<sup>9</sup>

#### EXPERIMENTAL

Our study of cholesterol methods was not begun with the idea of describing a new procedure, but rather with the idea of choosing the most suitable method for a problem based largely on blood cholesterol estimation. In Table I are shown some of the more important results obtained by a comparison of different extracting media, solvents, etc. The results given were chosen as representative of each particular study. The findings can be expressed very briefly as follows:

1. Whole blood or serum when dried on filter paper and extracted with chloroform gives values much lower than those obtained when the blood or serum is dried on plaster of Paris. The discrepancy in the two values is more marked with serum than with whole blood. This confirms the findings of both Bernhard and Dreker, and of Allardyce.

2. The amount of cholesterol extracted by chloroform from blood or serum dried on plaster of Paris is practically independent of the time or temperature of drying (within reasonable limits). About five minutes' time is sufficient, and the mixture may stand at least twelve hours at room temperature. However, about thirty minutes appears to be the optimum length of time. Drying at room temperature appears to give values almost identical with those obtained by drying at 100° C. and apparently the use of the electric oven is not required.

3. The cholesterol extracted by chloroform from blood or serum dried on filter paper varies with both the time and temperature of drying. Increases in temperature decrease the amount of sterols extracted in every case, if the time of drying is kept constant. Likewise if the temperature is kept constant, the cholesterol extracted decreases with the length of time of drying. Although Lieboff reports that the blood does not require drying before extraction, the author could not obtain satisfactory results in this manner.

4. The plaster of Paris employed must be fresh (not hydrated). Definitely lower values were obtained when hydrated plaster of Paris (hydrated by standing in the open air several days) was employed, especially when the blood and plaster was dried in the oven at 100° C.

# LABORATORY METHODS

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## A MODIFIED METHOD FOR THE DETERMINATION OF BLOOD CHOLESTEROL\*

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JEROME E. ANDES, PH.D., NEW ORLEANS, LA.

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IN THE determination of the cholesterol content of the blood and other body fluids, two general procedures are available. The first group of procedures comprises those in which the cholesterol is precipitated with digitonin, and estimated by either colorimetric or gravimetric means. In the second group the blood or serum is dried on a suitable medium, the cholesterol (and other fatty substances) extracted with a suitable fat solvent, and the cholesterol estimated colorimetrically by the Liebermann-Burchard reaction. The digitonin method, either as described by Windaus,<sup>1</sup> or according to one of the legion of modifications published in recent years, is probably the most accurate and reliable. However, it is quite an expensive method, and each determination requires considerable time for its completion. In addition, a fair degree of technical skill is needed for such analyses, and the average clinical technician does not possess a great amount of chemical laboratory training.

For this and other reasons the colorimetric method has become quite generally employed in clinical laboratories. The method is inexpensive, it requires a minimum amount of technical skill, and it requires much less time for each determination than does any of the digitonin methods. Also the values obtained are sufficiently accurate for clinical work.

Although Bloor<sup>2</sup> described the first direct colorimetric method, applicable to clinical work, the method of Myers and Wardell<sup>3</sup> published two years later was the first method that seemed to give satisfactory results in the hand of technicians. In this method the blood or serum is dried on plaster of Paris (at 100° C. for an hour), the cholesterol extracted with chloroform and the cholesterol in the chloroform extract determined colorimetrically by means of the Liebermann-Burchard reaction. This method, or as modified by Oser and Karr,<sup>4</sup> has proved to be very reliable, and is in use in many clinical laboratories at the present. The chief objections that may be offered to this method are the following: it requires apparatus not always present in clinical laboratories; at least 30 c.c. of chloroform are required for each determination; and the chloroform extract must be transferred to another vessel before making the colorimetric estimation. Lieboff<sup>5</sup> sought to simplify the method by the use of a special tube in which both the extraction and colorimetric estimation are made. Also he

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\*From the Department of Biochemistry, Louisiana State University Medical Center.  
Received for publication, April 9, 1934.

that extracted by chloroform. Also the results obtained by the use of alcohol-ether as a fat solvent were more erratic than when chloroform was employed.

Other desiccating media (as fuller's earth, powdered calcium carbonate and other inert salts, and zinc dust) were tried but found to be inferior to plaster of Paris. Also other extracting media (carbon tetrachloride, absolute alcohol, ether, etc.) were tested but again found to be similarly less desirable than chloroform.

From the above results it would seem that the most satisfactory method would be one employing plaster of Paris as a drying medium and chloroform as an extraction medium. While the method of Myers and Wardell satisfies these requirements, a micromodification of this method is described below which employs an extraction apparatus (composed chiefly of a Folin-Wu tube) in which the colorimetric comparison is also made.

#### METHOD FOR THE DETERMINATION OF CHOLESTEROL IN WHOLE BLOOD OR SERUM

*Principle.*—The blood (or serum) is mixed with plaster of Paris and allowed to dry a suitable length of time at room temperature. The cholesterol is then extracted by means of a simple refluxing apparatus and the colorimetric comparison carried out in the same tube.

*Procedure.*—Accurately pipette\* 0.25 c.c. of whole blood or serum into a 3-inch evaporating dish. Add a little over one-half teaspoonful of plaster of Paris (fresh) and thoroughly mix† with a spatula (a round-bottomed porcelain spoon is best used for this mixing). Scrape the plaster loose from the dish with a clean dry steel spatula, and cover the mixture with a watch glass and allow it to stand at room temperature for thirty minutes or longer.‡ Then by means of a "V"-shaped trough of smooth heavy paper, transfer the plaster of Paris to an extraction thimble of such a size that it will fit inside of a Folin-Wu tube§ (see Fig. 1). Place the extraction thimble inside the Folin-Wu tube, and arrange the rest of the extraction apparatus\*\* as shown in Fig. 1. Fill the bulb of the Folin-Wu tube about two-thirds full with pure chloroform, and place the tube on the hot plate. Add more chloroform as needed (until the thimble and contents become saturated with the chloroform) and carry on the extraction for at least thirty minutes. At the end of this time remove the condenser and extraction thimble and allow about one-half of the chloroform in the bulb to boil away.††

\*This is conveniently done by using a calibrated 1 c.c. pipette (graduated to 1/100 c.c.). The delivery between two marks is most satisfactory (i.e., between the 0.7 c.c. and 0.95 c.c. marks on the pipette).

†Thorough mixing is essential for accurate values.

‡It may be heated in an oven at 100°C. for an hour, or allowed to stand overnight at room temperature if desired.

§These thimbles can be purchased on the market, or can be conveniently made from library paste and thick filter paper. The thimble is molded over a small test tube, and after drying extracted with chloroform. The thimbles can be used for an indefinite number of determinations as the plaster of Paris is easily shaken out of the dry tube after each determination.

\*\*The apparatus is similar to the one described by Bernhard and Dreker. It chiefly consists of a Folin-Wu tube where 5 c.c. of volume comes in the constricted portion. This point is marked with a sharp file. The condenser is merely a test tube and is of such a size that its rim will just prevent its falling into the Folin-Wu tube (as shown); it is best made from a Pyrex test tube of a suitable size, cut off at the proper length.

††This removes any water that might have collected during the extraction.

TABLE I

STUDIES ON THE DETERMINATION OF CHOLESTEROL IN WHOLE BLOOD AND SERUM  
(Fig. in mg. per 100 c.c.)

COMPARISON OF PLASTER OF PARIS AND FILTER PAPER AS DESICCATING MEDIUM			EFFECT OF TIME AND TEMPERATURE WHEN USING PLASTER OF PARIS AS A DESICCATING MEDIUM		
SAMPLE NO.	VALUE	MEDIUM	SAMPLE NO.	VALUE	TIME AND TEMPERATURE
1. (Blood)	180	Plaster of Paris	7. (Serum)	213	30 min. at 25° C.
	181	Plaster of Paris		219	12 hr. at 25° C.
	170	Filter paper	8. (Serum)	110	30 min. at 25° C.
	166	Filter paper		109	12 hr. at 25° C.
2. (Blood)	198	Plaster of Paris	9. (Serum)	200	5 min. at 25° C.
	196	Plaster of Paris		199	30 min. at 25° C.
	136	Filter paper		201	30 min. at 25° C.
	133	Filter paper		199	12 hr. at 25° C.
3. (Blood)	193	Plaster of Paris	10. (Serum)	200	12 hr. at 25° C.
	132	Filter paper		123	30 min. at 25° C.
4. (Blood)	141	Plaster of Paris		119	1 hr. at 100° C.
	125	Filter paper	11. (Serum)	121	30 min. at 25° C.
5. (Serum)	135	Plaster of Paris		120	1 hr. at 100° C.
	80	Filter paper	12. (Serum)	1-1	30 min. at 25° C.
6. (Serum)	148	Plaster of Paris		180	1 hr. at 100° C.
	81	Filter paper			

EFFECT OF TIME AND TEMPERATURE WHEN USING FILTER PAPER AS A DESICCATING MEDIUM			NEW VS. OLD PLASTER OF PARIS (DRIED 1 HR. AT 100°)		
SAMPLE NO.	VALUE	TIME AND TEMPERATURE	SAMPLE NO.	NEW	OLD
13. (Blood)	170	30 min. at 25° C.	16. (Serum)	201	108
	151	8 hr. at 25° C.		199	105
	122	1 hr. at 100° C.	17. (Serum)	213	161
14. (Blood)	136	30 min. at 25° C.		219	152
	115	8 hr. at 25° C.	18. (Blood)	213	184
15. (Blood)	133	30 min. at 25° C.			
	110	30 min. at 40° C.			

*Comparison of Chloroform vs. Alcohol-Ether as an Extracting Medium*

SAMPLE NO.	CHLOROFORM (FROM PLASTER OF PARIS)	ALCOHOL-ETHER (FROM FILTER PAPER)	SAMPLE NO.	SERUM DRIED ON PLASTER OF PARIS	
				CHLOROFORM	ALCOHOL-ETHER
19. (Serum)	278	260	23. (Serum)	161	160
20. (Serum)	146	81		160	145
	142	76	24. (Serum)	178	165
21. (Serum)	200	148	25. (Serum)	121	120
	196	189			
22. (Serum)	180	165	26. (Serum)	221	217
	177	154			

5. The alcohol-ether mixture does not completely extract the sterols from blood or serum dried on filter paper,\* but when the blood is dried on plaster of Paris the amount extracted is similar (although slightly below in every case) to

\*We do not believe that this is necessarily contradictory to the findings of Bernhard and Drekter. The atmosphere in New Orleans is so nearly saturated with water vapor that a condensation of moisture takes place with any method employing an extraction apparatus. At the end of the period of extraction (with Bernhard and Drekter's method) from  $\frac{1}{2}$  to 1 c.c. of water had collected in the extraction tube. This had mixed with the alcohol-ether mixture, rendering it inefficient as a fat solvent. On a few days, when the atmosphere was somewhat less humid than usual, we were able to get results with filter paper and alcohol-ether, that closely approached those with plaster of Paris and chloroform. However, another big objection to the use of alcohol-ether is that it must be evaporated off and chloroform added before the colorimetric estimation. This evaporation requires considerable time, unless facilitated by a current of air. With the chloroform extraction the evaporation is not necessary.

2. A method is described for the determination of cholesterol in blood (or other body fluids). The blood is dried on plaster of Paris, and the cholesterol is extracted with chloroform, the entire extraction and color development being completed in a Folin-Wu sugar tube.

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Then remove the Folin-Wu tube from the hot plate, allow it to cool and add chloroform to the 5 c.c. mark. Now add 5 c.c. of the standard solution of cholesterol\* (containing 0.4 mg. cholesterol) into another Folin-Wu tube and add to each 1 c.c. of pure acetic anhydride. Now place both tubes in a beaker of water (at about 25° C.), and add 0.2 c.c. (5 drops) of concentrated sulphuric

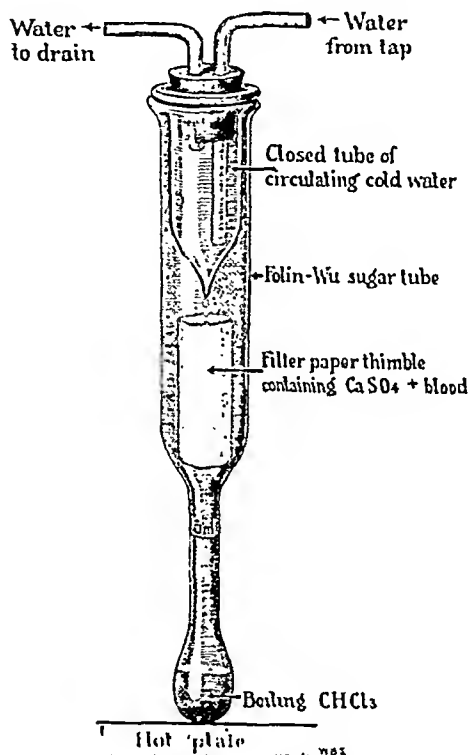


Fig. 1.—Cholesterol extraction apparatus.

acid. Mix† quickly, stopper tubes, and place the beaker and tubes in the dark. At the end of fifteen minutes compare the colors in a colorimeter.

*Calculation.*—The cholesterol in the blood or serum is calculated as follows:

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 160 = \text{mg. cholesterol per 100 c.c.}$$

The total time for a complete determination is about ninety minutes.

#### SUMMARY

1. In the colorimetric estimation of blood (or serum) cholesterol, plaster of Paris seems to be the most satisfactory desiccating medium and chloroform appears to be the most suitable extracting medium.

\*The stock standard is prepared by dissolving 160 mg. of pure cholesterol in 100 c.c. of chloroform. The working standard is made by pipetting 5 c.c. of this solution into a 100 c.c. volumetric flask, and making the volume up to 100 c.c. with chloroform. 5 c.c. = 0.4 mg. of cholesterol.

†The mixing is easily accomplished by blowing a current of air into the bottom of the tube. The air is dried by interposing a calcium chloride drying tube.



tions of formaldehyde, sodium citrate, Kemp and Calhoun's solution,<sup>12</sup> Dameshek's solution,<sup>13</sup> and the solution of Rees and Ecker.<sup>14</sup> The older physiologic solutions of Locke and Ringer, and the later modifications by Hédou and Fleig,<sup>15</sup> Adler<sup>16</sup> and Tyrode<sup>17</sup> were also used as diluents. The last named solution, with slight modifications, was used by Flössner<sup>18</sup> and Hofmann<sup>19</sup> for platelet counting. As diluents the physiologic solutions were found to be very unsatisfactory because they preserve the platelets for only fifteen to twenty minutes and because they either contain preformed artefacts or produce them on contact with blood. A preserving fluid, to be satisfactory, must answer the following requirements: (1) It must preserve the platelets for a reasonable length of time, at least several hours; (2) it must preserve the other cellular elements of the blood, the erythrocytes and leucocytes; (3) it must be isotonic so that the formed blood elements are not distorted; (4) it must be free from particulate matter; and (5) it must not produce precipitates or colloidal bodies when mixed with blood. The sodium metaphosphate solutions, first used by Pratt<sup>20</sup> for preserving and enumerating platelets, in general were found to be more satisfactory than any of the other diluting fluids. That sodium metaphosphate is an excellent preservative for blood platelets as well as the other cellular blood elements has been pointed out by many investigators (Deetjen,<sup>21, 22</sup> Hirschfeld,<sup>23</sup> Sabbatani,<sup>24</sup> Helber,<sup>25</sup> Pratt,<sup>26</sup> Richardson,<sup>27</sup> Cadwalader,<sup>28</sup> Gottlieb,<sup>29</sup> Aynaud,<sup>30</sup> Reid,<sup>31</sup> Maixner and von Decastello,<sup>32</sup> Degkwitz,<sup>33, 34</sup> Schenk,<sup>35</sup> Wittkower,<sup>36</sup> and Bannerman.<sup>37</sup>). The following solution was empirically found to be the most satisfactory:

	GM. OR C.C.
Sodium metaphosphate (Howe and French)	1.0
Sodium chloride	0.5
Dextrose	0.1
Distilled water	100.0

In this solution the platelets appear as clear, isolated, highly refractile bodies with numerous fine spinelike processes projecting from the periphery. The erythrocytes and leucocytes are excellently preserved; crenated or distorted red cells are rarely seen. It was found entirely superfluous to employ a dye to stain the platelets. However, if it is desirable to stain them, the following formula may be used:

	GM. OR C.C.
Sodium metaphosphate	1.0
Sodium chloride	0.4
Dextrose	0.1
Sodium bicarbonate	0.1
Brilliant cresyl blue	0.15
Distilled water	100.0

In this solution both the platelets and reticulocytes are well stained so that, as has been suggested by Dameshek,<sup>13</sup> a reticulocyte and thrombocyte count can be done simultaneously. The solutions, if not in use, should be kept in a cold place and should be filtered every few days.

#### TECHNIC FOR ENUMERATING THE BLOOD PLATELETS

The technic for counting blood platelets is more difficult than that of any other cellular constituent of the blood in view of the readiness with which they

## THE ENUMERATION OF BLOOD PLATELETS\*

ISADORE OLEF, M.D., BOSTON, MASS.

THE third formed cellular elements of the blood, the blood platelets or thrombocytes, were discovered by Donné<sup>1</sup> in 1842 who called them "globulins." Several years later they were independently observed by Zimmermann<sup>2, 3, 4</sup> who applied to them the term "Elementarkörperchen" or elementary corpuscles. The classical descriptions of these bodies by Schultze,<sup>5</sup> Osler,<sup>6, 7</sup> and particularly by Bizzozero<sup>8, 9</sup> placed them on an important hematologic basis. Bizzozero who described these cellular elements during life as they appeared in the mesenteric vessels of rabbits and guinea pigs, introduced the term "blood platelet."

The blood platelet count and its variations occupy an important part in hematology in view of the rôle these bodies play in blood coagulation, thrombus formation and also possibly the defense of the organism against infection by microorganisms. The platelets are probably the most susceptible of any of the formed blood elements to mechanical or chemical influences, and are quickest to regenerate when favorable conditions supervene (Doan and Sabin<sup>10</sup>). The enumeration of the thrombocytes is, therefore, important in the study of various hemorrhagic diseases, particularly purpura hemorrhagica; it probably is of greater significance than is realized in various anemias, leucemias and infections.

Numerous methods for counting blood platelets have been introduced since the time of Bizzozero. The difficulties encountered by various investigators in counting platelets are undoubtedly due to the peculiar physical properties of these cellular elements; viz., their great tendency to agglutination, adhesion, and easy disintegration under extravascular conditions. To these must be added their small size and light weight. Although the number of methods for enumerating the thrombocytes is large, none is fully satisfactory.

In 1930, I<sup>11</sup> described an indirect method for counting blood platelets, the diluting fluid consisting of a 2 per cent solution of sodium metaphosphate (Merck). Shortly after the publication of my method it was discovered that Merck & Co. had discontinued the manufacture of this chemical. A 2 per cent solution of a soluble polymer of sodium metaphosphate prepared by Howe and French of Boston was found to be too hypertonic for platelet counting. Some twenty different concentrations of this chemical, alone and in combination with other substances, were then experimented with. In addition a number of solutions that have been recommended for platelet counting were tried: physiologic saline, 14 per cent solution of magnesium sulphate, several different concentra-

\*From the Medical Clinic of the Boston Dispensary, Service of Dr. Joseph H. Pratt, and the Department of Medicine, Tufts Medical School.  
Received for publication, April 21, 1934.

This study was made possible by a grant from the Bingham Associates Fund.

on the theory that each platelet possesses a negative electric charge whose magnitude depends on a number of factors, as the hydrogen ion concentration of the blood or the composition of the blood plasma. An excess of substances like fibrinogen or globulin causes a diminution in the electric charge, thus favoring agglutination and disintegration of the platelets (Starlinger and Sametnik,<sup>53</sup> Stuber and Lang<sup>54</sup>). This is further favored by contact with foreign substances and chemical injury of various types (Dietrich<sup>55</sup>). The other is based on the theory originally proposed by Aehard and Aynaud<sup>56</sup> and further elaborated by Roskam<sup>57</sup> and Govaerts<sup>58, 59, 60, 61</sup> that there is adsorbed on the surface of each platelet a thin layer of plasma colloids, the so-called "plasma layer," which brings about agglutination and disintegration of platelets when the latter come in contact with water-wettable surfaces or foreign bodies. Osterhont<sup>62</sup> points out that when animal tissues are crushed or injured so that the internal fluid of the cells escapes, the fluid thus liberated may have a deleterious action on the healthy cells.

It is obviously important to bring the blood platelets rapidly in contact with the preserving fluid in order to obviate the destructive effects incident to the admixture of tissue juices, disintegration products of injured cells and of foreign bodies. This can be accomplished by (1) thorough cleansing of the skin before the puncture, (2) discarding the first drop or two of blood, (3) placing a drop of the preserving fluid over the puncture wound before the blood reaches the surface of the skin, and (4) mixing the blood and preserving fluid in paraffin or amber vessels. The discarded blood contains disintegration products of crushed and injured cells and is also diluted by the admixture of lymph; the subsequent drops of blood contain no such disintegration products and no admixture of lymph, since the higher pressure within the blood vessels very promptly forces the blood into the open lymph channels. Moreover, Pemberton,<sup>63</sup> Pierce and Pemberton<sup>64</sup> and Cronter and Cajori<sup>65</sup> have shown that in a considerable percentage of normal persons the first drop of blood yields higher erythrocyte counts than the subsequent drops; the reverse is true, they claim, in patients with arthritis. Obviously, when the indirect methods for counting platelets are employed where the erythrocyte-platelet ratio is determined, the first drop of blood should be discarded in order to obtain standard and comparable results. Horwitz<sup>41</sup> obtained higher platelet counts in the first drop of blood than in the subsequent drops; Steinmaurer<sup>66</sup> obtained no regular variations in the number of thrombocytes in the first and the fourth to fifth drops, although his average counts reveal somewhat greater numbers in the fourth to fifth drops of blood than in the first drop. In my own counts the first drop of blood has always yielded lower platelet values than the subsequent drops. The puncture wound should always be made sufficiently deep to obtain a fairly rapid flow of blood without the application of pressure. Bannerman<sup>37</sup> has shown that blood flowing rapidly yields higher platelet counts than blood flowing slowly. After the first drop or two of blood has been wiped away the further flow of blood can be easily controlled by gentle pressure over the wound with a piece of sterile gauze until the preserving fluid is applied.

disintegrate outside the body and the way they adhere to injured vascular epithelium and to foreign substances. The important technical steps in counting platelets are: (1) Drawing of the blood, (2) diluting and preserving the blood and (3) properly enumerating the platelets in the diluted blood. The first two procedures will be considered now.

#### OBTAINING BLOOD FOR PLATELET COUNT

According to Schenk,<sup>35</sup> Zeller,<sup>38</sup> Pagniez and Mouzon,<sup>39</sup> Kristenson,<sup>40</sup> Horwitz<sup>41</sup> and Jürgens<sup>42</sup> capillary blood is as accurate as venous blood for platelet counting and is certainly easier to obtain. A number of investigators (Aynaud,<sup>30</sup> Aehard and Aynaud,<sup>43, 44</sup> Thomsen,<sup>45</sup> Buckman and Hallisey,<sup>46</sup> Cramer and Bannerman,<sup>47</sup> Gutstein,<sup>48</sup> Preiss,<sup>49</sup> and Roskam<sup>50</sup>) recommend the use of venous blood in order to avoid admixture of tissue juices and the adhesion of platelets to raw surfaces. This, however, is easily obviated by puncturing the finger and following the procedure to be described. Moreover, the methods employing venipuncture involve too much manipulation of the blood and do not yield higher counts than the methods in which capillary blood is used.

The finger should always be used for drawing blood. It is advisable to employ a warm hand-bath immediately before puncturing the finger. This is carried out by immersing the hand in warm water, the hand being opened and closed for exercise. The active hyperemia thus produced accelerates the local circulation, facilitating a rapid flow of blood when the finger is punctured. The palmar surface of the finger tip is punctured with a Frank automatic lancet about one centimeter from the end after thorough cleansing of the parts with soap and water and subsequent drying with alcohol and ether or acetone. The lobe of the ear is not a satisfactory site for drawing blood; here the external temperature is apt to produce considerable changes in the capillaries, as has been pointed out by Naegeli.<sup>51</sup> Moreover, the presence of fine hair in that region favors the adhesion and agglutination of platelets. I<sup>11</sup> have shown that blood obtained from the finger yields higher platelet counts than blood obtained from the lobe of an ear.

#### PRESERVING AND DILUTING THE BLOOD

Extravascular changes in thrombocytes have been studied by a number of investigators, but just what the earliest changes are has never been determined. It is generally conceded, however, that the early changes are hastened by the admixture of tissue juices and retarded by the addition of anticoagulants and by dilution. When the blood platelets come in contact with a water-wettable surface, they become adherent to it, spread out, and rapidly disintegrate, liberating thromboplastic substances which are capable, in the presence of calcium salts, of transforming fibrinogen into fibrin with the formation of thrombin. If the water-wettable surface is smaller than that of the platelets, as in the case of colloids or microorganisms, the platelets attach themselves to it without disintegrating rapidly. The two important physicochemical processes involved in some of these changes are discussed by Morawitz and Brugsch.<sup>52</sup> One is based

fairly free flow of blood without applying pressure. The first drop or two of blood is wiped away; if the blood should form a dry layer on the surface of the skin, it may be washed off with a little of the diluent. A drop of the diluting fluid is then placed over the puncture wound before the blood reaches the surface of the skin and the hand quickly turned over so that the palmar surface is directed downward. The blood, having a greater specific gravity than the diluting fluid, falls rapidly to the bottom to the drop of diluent, as shown in Fig. 1. After a sufficiently large drop of blood has escaped, the entire mixture

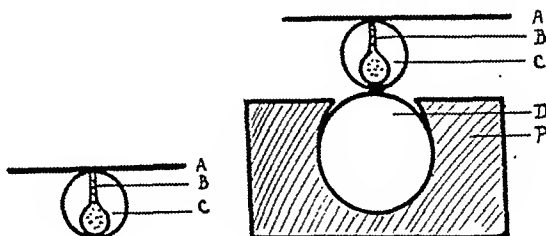


Fig. 1.

Fig. 2.

Fig. 1.—Technic for initial dilution of blood.

Fig. 2.—Technic for final dilution of blood. A, palmar surface of finger; B, drop of blood; C, drop of diluent; D, diluent in paraffin cup; P, paraffin cup.

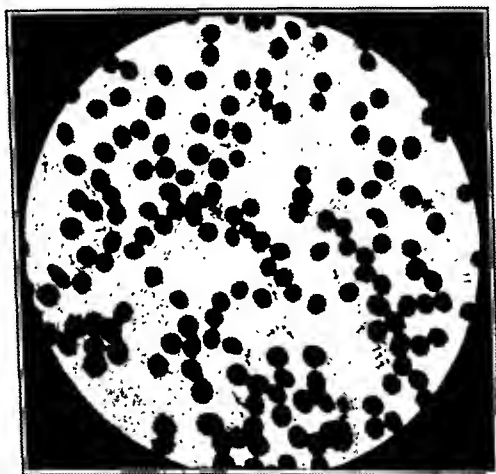


Fig. 3.—Microphotograph of a wet preparation from a normal blood ( $\times 800$ ) showing the ideal proportion of erythrocytes and platelets (the latter marked by arrows).

is applied to the surface of a small quantity (three to four drops) of diluting fluid contained in a paraffin cup, as shown in Fig. 2. The entire drop on the finger consisting of approximately equal parts of blood and diluent drops off into the cup. In this manner the blood is diluted approximately 1:5. The cup is prepared by melting the center of a small eube of paraffin, about 2 cm. on the side, with the heated end of a glass rod. The contents of the cup are then stirred gently with a wooden applicator, the end of which is coated with paraffin. The mixture is allowed to stand for a minute or two, stirred again and then is transferred by means of a clean paraffin-coated applicator to a glass slide;

Blood thus obtained from the finger must be mixed in the proper proportion with some diluent which not only preserves the platelets, but also the other cellular constituents of the blood. The use of undiluted blood for platelet counting, recommended by Jedlička and Altsehuller,<sup>67</sup> is undesirable because undiluted blood yields preparations too thick for accurate thrombocyte enumeration. Pratt,<sup>26</sup> Reid,<sup>31</sup> Sooy and Laurens<sup>68</sup> and Cumings<sup>69</sup> in their indirect methods, and Kemp and Calhoun,<sup>12, 70</sup> Flössner,<sup>18</sup> Hofmann,<sup>19</sup> Degkwitz,<sup>33, 34</sup> Schenk,<sup>35</sup> Wittkower,<sup>36</sup> Pagniez and Mouzon,<sup>39</sup> Achard and Aynaud,<sup>43</sup> Roskam,<sup>50</sup> Kemp,<sup>71</sup> Kemp, Calhoun and Harris,<sup>72</sup> Laker,<sup>73</sup> Muir,<sup>74</sup> Brodie and Russell,<sup>75</sup> and Determann<sup>76</sup> in their modified indirect methods allowed a drop of blood to fall into the diluting fluid placed on a glass slide or contained in a special vessel. This procedure carries with it considerable error in that the undiluted blood is allowed to come in contact with the surface of the skin and with the external air. My previous method involved this error. Bizzozero,<sup>8, 9</sup> Bannerman,<sup>37</sup> Zeller,<sup>38</sup> Pizzini,<sup>77</sup> Sahli,<sup>78</sup> Fonio,<sup>79</sup> Evans,<sup>50</sup> and Mackay<sup>81</sup> punctured the finger through a drop of diluting fluid; by this procedure obviously only the first drop of blood is available, and this does not yield accurate platelet counts, as has been pointed out before. Steinmaurer<sup>66</sup> and Hittmair<sup>82</sup> produce a superficial puncture without drawing blood, place a drop of the diluting fluid over the wound and by pulling the edges of the puncture apart allow some blood to escape into the overlying drop of diluent. It is not possible to pull the edges of the wound apart without exerting pressure in the neighborhood of the puncture, a procedure involving considerable error in platelet counting. Moreover, the use of the first drop of blood increases this error.

Bizzozero,<sup>8, 9</sup> Bannerman,<sup>37</sup> Steinmaurer,<sup>66</sup> Sahli,<sup>78</sup> Fonio,<sup>79</sup> Mackay,<sup>81</sup> Hittmair,<sup>82</sup> and von Boros and Kalstein<sup>83</sup> mixed the blood and the diluent on the finger. This technic involves a number of inaccuracies. During the process of stirring the mixture there is unavoidable contact of blood and skin with resulting destruction or clumping of platelets. If the blood is flowing freely, as it should if a correct count is to be obtained, the blood and diluting fluid form a very large drop in which it is rather difficult to obtain a uniform distribution of the blood and which frequently rolls off the finger, especially in women in whom the surface at the tip of the finger is small. Furthermore, freely flowing blood mixed with only one drop of preserving fluid yields preparations too thick for accurate platelet counting. Dameshek<sup>13</sup> does not stir the blood-diluent mixture at all; he places a drop of the preserving fluid over the puncture wound after the first drop or two of blood has been wiped away, then allows some blood to escape into the overlying drop of diluent and by applying a cover slip to the mixture, carries some of it away. This procedure is inaccurate because the platelets, being very light, quickly rise to the surface of the drop of diluting fluid before the considerably heavier erythrocytes have become uniformly distributed. The fluid on the cover slip, therefore, contains a relatively larger number of platelets than red cells.

The following procedure, in my opinion, obviates all the above mentioned inaccuracies in technic. The palmar surface of the finger tip is prepared in the manner previously described. A puncture is made sufficiently deep to obtain a

The minimum count for the group was 437,000 platelets per cubic millimeter; the maximum, 586,000. The average for the group was 514,000 platelets per cubic millimeter; the average for males was 525,000; that for females was 504,000.

In order to test the accuracy of the method, platelet counts were done simultaneously from two different fingers of the same person. The results are given in Table II.

The number of thrombocytes determined under the same physiologic conditions at the same hour of the day remains fairly constant. This is shown in Table III which represents the comparative platelet counts obtained from the same person on different days.

TABLE II  
COMPARISON OF PLATELET COUNTS FROM TWO DIFFERENT FINGERS ON THE SAME PERSON

SEX	AGE	PLATELETS PER C.M.M. FINGER I	PLATELETS PER C.M.M. FINGER II	PERCENTAGE OF VARIATION	REMARKS
M	35	434,000	436,000	0.4	Case of pernicious anemia under liver treatment
F	31	439,000	438,000	0.2	Chronic secondary anemia
F	33	637,000	635,000	0.2	Polycythemia
F	40	557,000	543,000	2.5	Catarrhal jaundice
F	16	580,000	590,000	1.7	Bronchial asthma
F	32	450,000	428,000	5.0	Chronic secondary anemia
M	35	565,000	575,000	1.8	Normal
M	32	519,000	536,000	3.2	Normal
F	40	564,000	563,000	0.2	Normal
M	35	517,000	511,000	1.1	Normal
M	28	500,000	495,000	1.0	Normal
F	41	547,000	545,000	0.4	Normal
M	25	583,000	558,000	4.4	Normal
M	33	504,000	496,000	1.6	Normal
M	45	537,000	505,000	6.1	Normal
M	33	482,000	494,000	2.7	Normal

TABLE III  
VARIATION OF THE PLATELET COUNT IN ADULTS

SEX	AGE	DATE (1933)	PLATELETS PER C.M.M.	AVERAGE	PERCENTAGE OF VARIATION	REMARKS
F	20	Oct. 19	451,000			
		Oct. 20	474,000	462,000	5.0	Normal
M	40	Nov. 16	452,000			
		Nov. 17	467,000	459,000	3.0	Mild chronic secondary anemia
M	36	Nov. 3	502,000			
		Dec. 23	494,000	498,000	1.6	Normal
M	37	Oct. 5	581,000			
		Dec. 21	560,000	570,000	3.7	Normal
M	34	Oct. 21	495,000			
		Dec. 19	480,000	487,000	3.0	Normal
M	25	Oct. 26	571,000			
		Dec. 15	578,000	574,000	1.2	Normal
F	22	Sept. 26	538,000			
		Dec. 21	508,000	523,000	5.7	Normal

#### DISCUSSION OF METHODS EMPLOYED FOR COUNTING THE BLOOD PLATELETS

The numerical estimation of the blood platelets has not come into general clinical practice apparently because simple methods of estimation are not suf-

usually three preparations can be obtained as the fluid in one cup yields three large drops. A cover slip is placed over each drop and after the preparations have been allowed to stand for ten to fifteen minutes, a relative platelet erythrocyte count is made, using the oil immersion lens. The glassware must be scrupulously clean, for, as Fenn<sup>84</sup> showed, hemolysis is most marked on slightly soiled glassware and may occur so rapidly with unfixed or improperly fixed cells that accurate counts are rendered impossible. When hemolysis occurs, artefacts in the form of "Arnold bodies" nearly always appear. Each oil immersion field should contain 100 to 150 erythrocytes (Fig. 3). In such a preparation the platelets appear as clear, isolated, highly refractile bodies suspended in the fluid medium, and are either motionless or exhibit Brownian motion. In thin preparations both the platelets and erythrocytes are liable to trauma from pressure of the cover slip; moreover, the platelets are often seen clinging to the underlying glass slide and eventually losing their refractile appearance, spreading out and disintegrating into coarse granules easily mistaken for small platelets. This explains the unduly high platelet counts frequently obtained in very thin preparations. In thick preparations the red cells often cover some of the platelets which escape the count. The platelets and erythrocytes are both counted in fields taken at random in different parts of the three preparations, until at least 1,000 red cells have been seen. An erythrocyte count is then done in the usual manner, and the absolute number of platelets per cubic millimeter is determined.

Table I represents the platelet counts obtained by the method described here in thirty-eight normal persons, twenty-two males and sixteen females.

TABLE I  
NORMAL PLATELET COUNTS BY THE AUTHOR'S METHOD

MALES		FEMALES	
AGE	PLATELETS PER C.M.M.	AGE	PLATELETS PER C.M.M.
35	494,000	40	470,000
27	521,000	40	482,000
28	500,000	24	491,000
40	568,000	30	480,000
33	500,000	41	541,000
31	505,000	40	512,000
25	570,000	52	437,000
33	495,000	45	559,000
33	516,000	20	474,000
26	511,000	40	513,000
35	514,000	19	445,000
30	488,000	16	528,000
35	502,000	20	500,000
24	486,000	22	538,000
35	570,000	40	564,000
32	577,000	52	519,000
14	502,000		
38	581,000		
28	546,000		
32	552,000		
44	510,000		
30	540,000		



amber. The direct methods for counting blood platelets, therefore, with twenty-three possible sources of error, are totally unreliable.

*Indirect Methods.*—In the indirect methods either the graduated pipette alone, or both the pipette and counting chamber are dispensed with. The ratio of platelets to red cells is first obtained. Then, by doing an erythrocyte count in the usual manner, the absolute number of platelets per cubic millimeter is calculated. Three different indirect methods are being employed for platelet counting.

1. *Dry Indirect Methods:* Dried films are prepared from undiluted or diluted blood, stained with some suitable dye and the ratio of platelets to erythrocytes determined. Introduced by Rabi<sup>86</sup> in 1896, it was employed with some modifications by Steinmaurer,<sup>66</sup> Fonio,<sup>79</sup> Hittmair,<sup>82, 92</sup> Gottlieb,<sup>93</sup> Stahl,<sup>94</sup> Oseladore,<sup>95</sup> Leschke and Wittkower,<sup>96</sup> Gáspár<sup>97</sup> and Doenecke.<sup>98</sup> With the exception of Hittmair and Steinmaurer, the investigators using this method obtained normal counts of approximately 250,000 platelets per cubic millimeter. This method is not trustworthy; many of the platelets are destroyed during the preparation of the films, and some are washed off during the process of staining. Hittmair's modification of this method yields normal counts of about 480,000 platelets per cubic millimeter; that of Steinmaurer, 690,000. Some of the inaccuracies involved in the technic of these two investigators have been pointed out before. Moreover, Hittmair employs as diluent a 14 per cent solution of magnesium sulphate which is highly hypertonic and, according to Boshamer,<sup>99</sup> breaks up some of the platelets, thus raising the count. Steinmaurer used Tyrode's solution as the diluting fluid; the errors involved when this solution is employed as a preservative for platelets will be discussed later. Apparently the dry indirect methods, although simple in their application, possess many inherent sources of error and are, therefore, unreliable.

2. *Modified Indirect Methods:* In these methods the graduated pipette is discarded, the blood being mixed with the diluent on the finger, on a glass slide, or in a special vessel, and a drop of the mixture then placed in a counting chamber where the relative number of platelets and erythrocytes is determined. The results obtained by various investigators using this method are given in Table V.

TABLE V

AVERAGE NUMBER OF BLOOD PLATELETS PER CUBIC MILLIMETER OF NORMAL BLOOD AS DETERMINED BY VARIOUS AUTHORS USING THE MODIFIED INDIRECT METHOD

AVERAGE NUMBER OF PLATELETS PER C.M.M.	AUTHOR	AVERAGE NUMBER OF PLATELETS PER C.M.M.	AUTHOR
150,000 - 200,000	Sahli <sup>78</sup>	300,000	Evans <sup>80</sup>
210,000 - 245,000	Roskam <sup>80</sup>	300,000	Degkwitz <sup>33, 34</sup>
216,000	Achard and Aynaude <sup>43</sup>	400,000	Laker <sup>73</sup>
200,000 - 250,000	Muir <sup>74</sup>	590,000 - 760,000	Cummings <sup>69</sup>
227,000	Determann <sup>76</sup>	635,000	Brodie and Russell <sup>75</sup>
230,000	Schenk <sup>35</sup>	682,000 - 760,000	Flössner, <sup>18</sup> Hof- mann <sup>19</sup>
250,000	Bizzozero <sup>8</sup>	600,000 - 900,000	Horwitz <sup>41</sup>
233,000 - 388,000	Pagniez and Mouzon <sup>39</sup>	773,000 - 862,000	Kemp and Calhoun <sup>12, 70</sup>
250,000 - 300,000	Wittkower <sup>36</sup>		

ficiently accurate and because the more exact methods are not sufficiently simple. The great number of methods and modifications of methods found in the literature indicates a lack of standardization in technique. The methods thus far introduced for enumerating the blood platelets can be divided into three groups: direct methods, indirect methods, and special methods.

*Direct Methods.*—The direct methods for counting blood platelets involve the use of the graduated pipette and the counting chamber. The results obtained by various investigators employing this method are given in Table IV.

That the direct methods are untrustworthy is shown by the normal platelet counts reported which vary from 100,000 to 1,000,000 per c.mm. Many early investigators (Laker,<sup>73</sup> Muir,<sup>74</sup> Brodie and Russell,<sup>75</sup> Determann,<sup>76</sup> Pizzini,<sup>77</sup> Halla,<sup>85</sup> Rabl<sup>86</sup>) recognized the fallacies of the direct methods. Krumbhaar<sup>87</sup> enumerates sixteen common errors in counting erythrocytes. Everyone of these

TABLE IV  
AVERAGE NUMBER OF PLATELETS PER CUBIC MILLIMETER AS DETERMINED BY  
VARIOUS AUTHORS USING THE DIRECT METHOD

AVERAGE NUMBER OF PLATELETS PER C.M.M.	AUTHOR	AVERAGE NUMBER OF PLATELETS PER C.M.M.	AUTHOR
100,000 - 350,000	Oestreich <sup>140</sup>	297,000	Wright and Kinnicutt <sup>145</sup>
180,000 - 250,000	Fusari <sup>141</sup>		Afanassiow <sup>146</sup>
210,000	Aynaud <sup>142</sup>	200,000 - 300,000	Howlett <sup>147</sup>
228,000	Helber <sup>25</sup>	250,000 - 300,000	Goadby <sup>148</sup>
230,000 - 240,000	Port and Akiyama <sup>143</sup>	300,000	van Herwerden <sup>149</sup>
245,000	Gutstein <sup>48</sup>	337,000 - 340,000	Jepsen <sup>103</sup>
245,000	van Emden <sup>73</sup>	300,000 - 500,000	Preiss <sup>49</sup> ✓
250,000	Hayem <sup>88</sup>	320,000 - 450,000	Barbieri <sup>150</sup>
250,000	Gottlieb <sup>29</sup>	360,000 - 500,000	Prus <sup>151</sup>
251,000	Maixner and Decas- tello <sup>32</sup>	500,000	Casey and Helmer <sup>152</sup>
255,000	Ottenberg and Rosenthal <sup>144</sup>	536,000	Puchberger <sup>153</sup>
284,000	Buckman, and Hallisey <sup>46</sup>	600,000	Lampert <sup>91</sup>
		1,000,000	

errors is possible also when counting platelets by the direct methods in addition to the following: Clumping of the platelets during the drawing of the blood into the pipette, the impossibility of visualizing the smaller platelets with the high dry lens, the failure to visualize some of the platelets in the 100 microns space that exists between the bottom of the counting chamber and the overlying cover glass, the sticking of the platelets to the sides of the pipette and to the parts of the hemacytometer, and the confusion of foreign particles or precipitates with platelets. Even the temperature of the pipette in this method is apt to cause considerable error, as was pointed out by Kemp, Calhoun and Harris<sup>72</sup> and by Hayem;<sup>88</sup> in a warm room the error would be greater than in a cold room. A source of error frequently overlooked is the dilution of the blood. Windfeld<sup>89, 90</sup> showed that plasma diluted 1:20 will often give platelet counts twice as great as undiluted plasma, due to the fact that in higher dilutions the platelets disintegrate readily, yielding greater values. This is especially liable to occur when diluents are employed which preserve the platelets poorly, as Tyrode's solution. This source of error explains partly the extremely high normal values reported by Lampert<sup>91</sup> who employed a specially constructed pipette made of

(Eufinger and Knobloch<sup>106</sup>). Lampert<sup>105</sup> as well as Eufinger and Knobloch<sup>106</sup> also believe that some of these formations are colloidal bodies. Doenecke,<sup>98</sup> Jepsen,<sup>103</sup> Behr,<sup>104</sup> and Hartmann<sup>107</sup> consider these bodies to be artefacts whose nature is uncertain.

The small bodies above referred to are very similar in appearance and size to the formations described by Edelmann<sup>108</sup> as the fourth blood element and believed by Pelczar and Koloszynski<sup>109</sup> to represent microthrombocytes arising during the process of disintegration of platelets. Moreover, these small bodies are also quite similar, according to Blacher,<sup>110</sup> to hemokonia or blood dust first described by Müller;<sup>111</sup> although best seen when examined in the dark-field, they can at times be observed with the oil immersion lens in ordinary preparations. Hemokonia may arise from fat droplets in the blood (Schilling<sup>112</sup>), from blood platelets (Horwitz,<sup>41</sup> Boshamer<sup>99</sup>), and possibly from white blood cells (Behr<sup>104</sup>). The degeneration products of erythrocytes known as pseudospirochetes and filamentous forms ("Blutfäden") and described by Zeller,<sup>113</sup> Schultz,<sup>114</sup> Takeuchi,<sup>115</sup> von Neergaard,<sup>116</sup> Auer,<sup>117</sup> Andre,<sup>118</sup> and Doerr and Seidenberg,<sup>119</sup> rapidly break up with the formation of coccoid forms similar to hemokonia. This is particularly apt to occur in blood from febrile patients. Furthermore, the above mentioned small bodies are very similar to some of the smaller "Arnold bodies" (degeneration products of red cells) described by Schultze,<sup>5</sup> Osler,<sup>7</sup> Hayem,<sup>88</sup> Wlassow<sup>120</sup> and particularly by Arnold<sup>121</sup> and visible in ordinary oil immersion preparations. These minute bodies, in other words, are either artefacts or fragments of disintegrated platelets or of the other cellular blood constituents. They possess neither the morphology nor the staining characteristics of platelets. They cannot, therefore, be considered as thrombocytes.

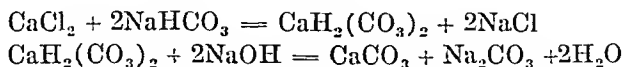
A characteristic feature in preparations where Tyrode's solution is employed as the diluent, is the presence of large numbers of small thrombocytes whose size is one-quarter the diameter of a red cell (1.8 microns) or less. Some investigators using this solution (Flössner,<sup>18</sup> Hofmann,<sup>19</sup> Horwitz,<sup>41</sup> Cumings,<sup>60</sup> Boshamer<sup>99</sup>) are of the opinion that in it the smaller platelets are preserved; these small forms, they believe, are very fragile and are apt to disintegrate rapidly, thus escaping the count. Jürgens<sup>42</sup> who employed a modification of Thomsen's<sup>45</sup> method, holds a similar view. In Tyrode's solution the small thrombocytes form 50 to 70 per cent of all of the platelets. The average size of normal platelets as reported by various observers not using Tyrode's solution, is given in Table VI.

TABLE VI  
AVERAGE DIAMETER OF PLATELETS AS DETERMINED BY VARIOUS AUTHORS

AVERAGE DIAMETER OF PLATELETS IN MICRONS	AUTHOR	AVERAGE DIAMETER OF PLATELETS IN MICRONS	AUTHOR
0.5 - 2.0	Dameshek <sup>13</sup>	2.0 - 3.0	Mackay <sup>81</sup>
2.0 - 5.0	Richardson <sup>27</sup>	3.0	Stahl <sup>94</sup>
2.0 - 3.0	Degkwitz <sup>33, 34</sup>	2.0 - 6.0	Kolozs <sup>100</sup>
2.0 - 3.0	Achard and Aynaud <sup>43</sup>	2.0 - 5.0	Reimann <sup>132</sup>
2.0 - 3.0	Preiss <sup>40</sup>	2.5 - 4.0	Howlett <sup>154</sup>
2.0 - 4.0	van Emden <sup>73</sup>	1.8 - 3.6	Afanassiew <sup>155</sup>
2.0	Muir <sup>74</sup>	3.0	Maximow and Bloom <sup>156</sup>
2.5 - 5.0	Determann <sup>76</sup>		

In the ordinary hemacytometer foreign bodies might be easily confused with platelets; hence the unreliability of counts obtained in this manner. Even when the dry high power lens is used, products of degenerated erythrocytes ("Arnold bodies"), foreign particles and bacteria may be mistaken for platelets. The platelets themselves, particularly the small forms, are often recognized with difficulty in the chamber. A serious objection to the use of the counting chamber is its depth, as was pointed out by Pratt,<sup>26</sup> Halla,<sup>55</sup> and others. The ordinary Thoma-Zeiss hemacytometer is 0.1 mm. or 100 microns deep, which is about forty times the diameter of the average platelet. The erythrocytes quickly settle to the bottom, but the platelets, being considerably lighter in weight, tend to float in the upper layers of the fluid and settle to the bottom very slowly. It is, therefore, difficult not to miss some of the platelets in the count. Even when specially constructed chambers with smaller depths are employed, methods involving their use are unreliable (Helber,<sup>25</sup> Pratt<sup>26</sup>). The counts obtained by the modified indirect methods, therefore, will be low if many of the platelets escape the count because of the impossibility of visualizing them all in the hemacytometer; or the counts will be too high if foreign particles and bacteria in the counting chamber are mistaken for platelets. Kemp and Calhoun's<sup>12, 70</sup> high counts, for example, are due to the inclusion in the platelet counts of "Arnold bodies" which are not readily differentiated from platelets with the dry high power lens.

The modified indirect method of Flössner<sup>13</sup> and Hofmann<sup>19</sup> where Tyrode's solution is used as the diluting fluid, has been adopted recently by a number of investigators (Horwitz,<sup>41</sup> Cummings,<sup>60</sup> Boshamer,<sup>99</sup> Kolozs,<sup>100</sup> Schulte<sup>101</sup> and Weiss<sup>102</sup>). Tyrode's solution is also the diluting fluid in the direct methods of Preiss,<sup>40</sup> Lampert,<sup>91</sup> Jepsen,<sup>103</sup> and Behr,<sup>104</sup> and in the dry indirect method of Steinmaurer.<sup>66</sup> This solution, which is a good artificial nourishing fluid, has the following composition: NaCl 8; KCl 0.2; CaCl<sub>2</sub> 0.1; NaH<sub>2</sub>PO<sub>4</sub> 0.05; NaHCO<sub>3</sub> 1; dextrose 1; oxygen to saturation, and H<sub>2</sub>O 1,000. When Tyrode's solution is used as a diluting fluid, numerous small, rounded bodies, approximately 0.5 to 1 micron in diameter, appear in the preparations. They are very infrequent in other preserving fluids. These bodies exhibit very active Brownian-like motions, changing their position continuously among the red cells. They do not stain readily with the ordinary dyes and appear to be highly resistant when in contact with glass, much more so than platelets. There has been considerable controversy as to the nature of these bodies. Horwitz,<sup>41</sup> Steinmaurer,<sup>66</sup> Cummings,<sup>60</sup> Boshamer,<sup>99</sup> Kolozs,<sup>100</sup> Schulte<sup>101</sup> and Weiss<sup>102</sup> consider them as platelets. Lampert<sup>105</sup> considers them as crystalline products formed by the interaction of the solution with glass, with the formation of CaCO<sub>3</sub>:



CaCO<sub>3</sub>, being slightly soluble, is precipitated out in the form of crystals. By preparing a special optically pure Tyrode's solution Lampert was able to reduce the number of these bodies, but there were still many present. They are present even after the solution has been carefully filtered ten to twelve times

ployment of Tyrode's solution as diluent which contains a variety of artefacts easily confused with platelets. This method for enumerating the thrombocytes is, therefore, untrustworthy.

3. *True Indirect Methods*: In these methods both the graduated pipette and the counting chamber are dispensed with. A drop of the diluted blood is placed on a clean glass slide, covered with a cover slip and by using the oil immersion lens the ratio of platelets and erythrocytes determined. These methods for counting blood platelets are, in my opinion, the most accurate because they involve a minimum manipulation of the blood. The results obtained by various investigators using this method are given in Table VIII.

TABLE VIII

AVERAGE NUMBER OF BLOOD PLATELETS PER CUBIC MILLIMETER AS DETERMINED BY VARIOUS AUTHORS USING THE TRUE INDIRECT METHOD

AVERAGE NUMBER OF PLATELETS PER C.M.M.	AUTHOR	AVERAGE NUMBER OF PLATELETS PER C.M.M.	AUTHOR
250,000	Bannerman <sup>37</sup>	327,000	Cadwalader <sup>123</sup>
300,000	Reid <sup>31</sup>	390,000	Mackay <sup>81</sup>
312,000	Cramer and Bannerman <sup>47</sup>	469,000	Pratt <sup>26</sup>
	von Boros and Kalstein <sup>83</sup>	590,000 - 760,000	Cummings <sup>69</sup>
300,000 - 600,000		619,000	Olef <sup>11</sup>
		716,000	Dameshek <sup>13</sup>

The considerable divergence in the results reported is due either to inaccuracies in the technique employed, or to the preserving fluid used as diluent. Bannerman's<sup>37</sup> technique is inaccurate because he used the first drop of blood and then mixed the blood and diluent on the finger. The procedures of Reid,<sup>31</sup> Cadwalader<sup>123</sup> and Pratt<sup>26</sup> are very similar and involve errors because the first drop of blood drawn is transferred to a drop of diluent on a glass slide, thus permitting the blood to come in contact with the surface of the skin and the external air. The method of Cramer and Bannerman<sup>47</sup> contains several errors: (1) too great manipulation of the blood which is obtained by venipuncture, (2) too great dilution of the blood (1:10), and (3) mixing blood and diluent with a teated pipette not covered with paraffin. Von Boros and Kalstein<sup>83</sup> as well as Mackay<sup>81</sup> used the first drop of blood and mixed the blood and diluting fluid on the finger; this procedure is inaccurate. Cummings<sup>69</sup> employed Tyrode's solution which is a very poor preserving fluid for platelets. My previous method<sup>11</sup> involved the error of allowing the blood to come in contact with the surface of the skin and the external air. Dameshek's<sup>13</sup> method is inaccurate because, as previously noted, the blood and diluent do not form a uniform mixture, thus yielding relatively high platelet counts.

*Special Methods.—Zeller's Method*: The method of Zeller<sup>38</sup> is essentially a modified indirect method and is too complicated for clinical purposes, as it involves the use of the torsion balance. By employing Kemp-Calhoun's<sup>12</sup> diluting fluid he obtained normal values of 500,000 to 750,000 platelets per cubic millimeter. The sources of error in this method are (1) too much handling of the blood, (2) the employment of the hemacytometer, and (3) the use of Kemp-

From this table it is obvious that the average size of normal platelets, according to most investigators, is 2 microns or greater; Dameshek's<sup>13</sup> smaller figures are due to the fact that his solution is hypertonic.

According to size, platelets are best classified into three groups: Group I, consisting of platelets whose size is one-quarter the diameter of a red cell, or about 1.8 microns; Group II, consisting of platelets one-third the diameter of a red cell, or about 2.5 microns; and Group III, consisting of platelets one-half the diameter of a red cell or greater, or about 3.6 microns. The occasionally present irregular forms may be placed in Group IV. Table VII represents the classification of platelets according to size, as determined in wet preparations by Boshamer,<sup>99</sup> Degkwitz<sup>33</sup> and by the author.

TABLE VII

DIFFERENTIATION OF PLATELETS ACCORDING TO SIZE AS DETERMINED IN WET PREPARATIONS

AUTHOR	GROUP I	GROUP II	GROUP III	GROUP IV	REMARKS
Boshamer	49%	43%	3.5%	4.5%	Tyrode's solution used as diluent
Boshamer	17%	74%	4.0%	5.0%	14% MgSO <sub>4</sub> solution used as diluent
Boshamer	18%	72.5%	4.5%	5.0%	Degkwitz's solution used as diluent
Degkwitz	-	94.4%	5.6%	-	Sodium metaphosphate used as diluent
Author	18.8%	72.2%	9.0%	-	Average results obtained on 18 normal persons. One per cent sod. metaphosphate used as diluent

Most platelets, apparently, are medium sized with an average diameter of 2.5 microns. Why is it, then, that when Tyrode's solution is used, a large percentage of the platelets are of the smaller variety, or Group I? The explanation is given by a number of investigators (Preiss,<sup>49</sup> Jepsen,<sup>103</sup> Lampert,<sup>105</sup> Hartmann<sup>107</sup>) who observed that in Tyrode's solution after a relatively short time, twenty to thirty minutes, the smaller platelets increase in number as the larger platelets diminish. Evidently the increase in the number of the smaller platelets is due to the disintegration of the larger forms. Preiss<sup>49</sup> proved this conclusively in his experiments by injecting hirudin into rabbits before drawing blood for platelet counting, in order to prevent extravascular disintegration of the thrombocytes. Although Tyrode's solution, which is a poor preservative for platelets, was employed as the diluting fluid, yet the number of small platelets, even at the end of one hour, remained approximately the same. Preiss,<sup>49</sup> Jepsen,<sup>103</sup> Behr,<sup>104</sup> Lampert<sup>105</sup> and Baedorf<sup>122</sup> who used Tyrode's solution as diluent, believing the diminutive platelets to represent disintegration products, excluded them from their counts.

The Flössner-Hofmann modified indirect method for platelet counting evidently involves errors due to the use of the counting chamber and to the em-

plasma which will yield consistent counts. In fact, following the introduction of the pipette into the plasma, the sudden streaming of the very light platelets from the lower levels of the plasma where they have become more numerous, to the upper levels will at times give higher counts in the upper layers even after the blood has been standing for several hours. By employing Reimann's<sup>132</sup> modification of Thomsen's technic and using a 1 per cent solution of sodium metaphosphate as diluent, I carefully removed with a pipette at hourly intervals a small quantity of plasma from the very surface of the plasma layer, taking great care not to produce any currents in the fluid. At the end of two hours the uppermost level of the plasma column contained 474,000 platelets per c.mm. of plasma; at the end of three hours there were 360,000 platelets per c.mm. in the same plasma level, and at the end of four hours there were 344,000 platelets per c.mm. Moreover, at the end of four hours there were 265,000 platelets per c.mm. in the center of the column of sedimented red cells. Apparently the platelets do not remain uniformly suspended in the plasma for several hours, as is assumed by those employing Thomsen's method, but are gradually gravitating to the lower levels of the column of plasma, the rate of sedimentation of the thrombocytes depending, according to Jürgens,<sup>42</sup> on their number, size, and the composition of the plasma. This has also been observed by Baunack<sup>135</sup> who showed that after one to two hours the concentration of the platelets in the lower layers of the plasma column may be from 40 to 50 per cent greater than their concentration in the upper levels. Moreover, even at the end of several hours there are still a good many platelets among the red cells.

According to Petri,<sup>133, 134</sup> Thomsen's macromethod involves an error of 10.4 per cent with undiluted plasma and an error of 21.9 per cent with diluted plasma. Thomsen's micromethod involves an error of 20.3 per cent according to Petri<sup>136, 137</sup> and an error of 8 to 10 per cent according to Als.<sup>138</sup> Petri's improved "centrifuge" modification<sup>133, 134</sup> of Thomsen's method has a minimum error of 7.1 per cent with venous blood and a minimum error of 9.4 per cent with capillary blood. Thomsen<sup>45</sup> found that after centrifuging the blood for ten minutes at 2,000 revolutions per minute there was still 5 per cent plasma among the red cells, and Gram<sup>139</sup> found that after centrifuging the blood for fifteen minutes at 3,000 revolutions per minute there still remained 5 to 10 per cent of plasma among the red cells.

If to the above numerous sources of error are added those inherent in any direct method for platelet counting, then Thomsen's method and its modifications become entirely untrustworthy.

#### SUMMARY

The most accurate procedures for counting the blood platelets are the true indirect methods. The true indirect method described here is, I believe, accurate and dependable, maximum care having been exercised in carrying out the various technical steps involved. It adheres to the leading principles that must be taken into consideration in counting platelets: (1) avoidance of contact between undiluted blood and skin, external air, glassware or metal, (2) mixing

Calhoun's solution which is a poor preserving fluid for both erythrocytes and platelets. "Arnold bodies," resulting from the disintegration of red cells, are readily formed in this fluid as is obvious from Kemp's<sup>124</sup> description of platelets containing hemoglobin. The platelets also disintegrate rapidly as is apparent from Zeller's<sup>38</sup> description of the platelets as varying in size from the diameter of a red cell to a point. This method is not reliable.

*Kristenson's Method:* The method of Kristenson<sup>40</sup> involves the use of a specially constructed syringe for obtaining venous blood and the subsequent use of the counting chamber. In 1928 he modified his method<sup>125</sup> by using capillary blood and a special graduated pipette. His method was adopted by Hartmann,<sup>107</sup> Baquero Gil,<sup>126</sup> and Lueia and Riekard.<sup>127</sup> Kristenson obtained normal values of 300,000 platelets per c.mm. with his first method, and from 326,000 to 424,000 platelets per c.mm. with his second method. Neither method is trustworthy for obvious reasons. The first is essentially a modified indirect method, the second a direct method. Moreover, the diluting fluid which consists of urea, sodium citrate, corrosive sublimate and cresyl violet is hypertonic and dissolves the erythrocytes (Kristenson,<sup>40</sup> Lueia and Riekard<sup>127</sup>).

*Thomsen's Method:* Thomsen's<sup>45</sup> method is based on the original observation of Bürker<sup>128</sup> that platelets remain suspended for a long time in undiluted plasma. Blood is obtained by venipuncture into a syringe containing a small amount of sodium citrate and allowed to stand until the red cells have settled. A small quantity of the supernatant plasma is then drawn into a white cell pipette, diluted with some suitable diluent and a platelet count done in the ordinary hemacytometer. By determining the volume of packed red cells the absolute number of platelets per cubic millimeter of blood can be obtained. Thomsen modified his procedure in 1923 by introducing his micromethod.<sup>129, 130, 131</sup> The results obtained by various investigators using this method, or modifications of it, are given in Table IX.

TABLE IX

AVERAGE NUMBER OF BLOOD PLATELETS PER CUBIC MILLIMETER AS DETERMINED BY VARIOUS AUTHORS USING THOMSEN'S METHOD

AVERAGE NUMBER OF PLATELETS PER C.M.M.	AUTHOR	AVERAGE NUMBER OF PLATELETS PER C.M.M.	AUTHOR
200,000 - 500,000	Gram <sup>139</sup>	348,900	Petri <sup>133, 134</sup>
230,000	Schenk and Spitz <sup>157</sup>	350,000	Reimann <sup>132</sup>
250,000 - 300,000	Thomsen <sup>45</sup>	352,000 - 531,000	Windfeld <sup>89, 90</sup>
270,000	Spitz <sup>158</sup>	650,000	Jürgens <sup>42</sup>
320,000	Nygaard <sup>159</sup>		

Those employing this method assume that the platelets remain uniformly suspended in the plasma even for as long as six hours. If the number of platelets per cubic millimeter is determined in various levels of the plasma at different intervals the results obtained will usually be fairly uniform. This is due to the fact that when a pipette is introduced into the plasma column for the purpose of drawing some of it off for a platelet count, currents are unavoidably produced in the fluid, mixing the lower and upper layers of the column of



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of freshly drawn blood as quickly as possible with the preserving fluid, and (3) avoidance of all unnecessary manipulations of the blood. It is simple to carry out and can be easily adapted for routine clinical use.

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and into the current of air steadily maintained by an air blower cannot be favored during both phases of respiration. In fact, the greater the assistance during one phase, the greater will be the hindrance during the other. For physiologic reasons, interference with inspiration however slight is always objectionable and readily becomes distressing. On the other hand, resistance to expiration, if slight, is readily and totally ignored by the subject.

In a well-conditioned Benedict-Roth apparatus, with unobstructed connections and properly cared for soda lime, there is no appreciable resistance to air flow induced in the entire circuit by the act of breathing only. The best that can be done is to favor inspiration only to such a slight degree as to impose a correspondingly negligible resistance during expiration. In the writer's opinion, *there is no better way, to insure ease and comfort in breathing, than by simply having the spirometer bell slightly overbalanced, thus favoring inspiration.*

Extensive observations (1920) on oxygen consumption, pulse and respiration rates, with valves in comparison with the electric air impeller<sup>4</sup> proved that the latter was no longer necessary because of the elimination from the circuit of all but one absorber and much tubing, thus reducing air resistance to a minimum. (Incidentally, the resistance offered to the passage of the expired air through an ordinary charge of well-sifted soda lime, 4 to 8 mesh, is about the same as when passing through one foot of three-quarter-inch tubing. It is, in fact, negligible.)\*

Recent substantial improvements, in compactness chiefly, insure still greater ease in breathing without the necessity of any troublesome mechanical contrivance of any kind.

Professor August Krogh, Copenhagen,<sup>5</sup> and the writer<sup>4</sup> published simultaneously (1922) but unknown to each other, their views in which both advocate the use of valves in the place of an electric blower.

On the other hand, a motor-blower of adequate efficiency is necessary when the apparatus is modified for allowing the use of a helmet as advocated by Benedict<sup>7</sup> or of such accessories as may impede the free circulation of air and impose any but negligible respiratory effort upon the subject.

The use of valves in the "Tissot" gasometer method has never been criticized. In fact, there is here no alternative, 100 per cent efficient valves are absolutely indispensable. The work imposed upon the subject in moving a well-compensated relatively heavy, 50 to 100 liter gasometer bell, has proved to be no appreciable impediment to breathing. There cannot, therefore, be any valid argument against the extremely negligible corresponding task in displacing the much smaller and lighter bell used in the "Benedict type" of apparatus.

In the twenty-two years during which in our service metabolism testing has been extensively carried on by means of every type of apparatus above referred to, our experience has been that a minimum of complaints is elicited when the quiet, valve apparatus is used.

Whether the valve or a motor-blower is used and no matter how carefully the test may be conducted patients will be encountered who dislike the ordeal

\*Measurements made with a manometer inserted through the wall of the rubber mouth-piece of a properly conditioned valve apparatus showed a maximum water pressure of only 3 or 4 mm. during ordinary expiration and 8 to 10 mm. during forced expiration. No appreciable effect was noted during normal inspiration and only 5 or 6 mm. of negative pressure during forced expiration.

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## VALVES VERSUS THE MOTOR-BLOWER\*

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IN THE BENEDICT TYPE OF METABOLISM APPARATUS NOW IN COMMON USE

---

PAUL ROTH, M.D., BATTLE CREEK, MICH.

---

THE first metabolism apparatus of the Benedict type, the Universal,<sup>1</sup> was necessarily motor operated because of its very complex air circuit. It included four moisture and carbon dioxide absorption chambers besides many feet of tubing through which the air was forced by means of a Crowell rotary air pump.

The next Benedict type, the "portable"<sup>2</sup> or "Benedict-Collins,"<sup>3</sup> was considerably simplified and was adequately ventilated by means of a small electric air blower.

I presented in 1922<sup>4</sup> a modification, the "Benedict-Roth," in which the very simple "Sadd" valve was installed and the air blower discarded. The elimination of the latter was greeted with general favor and valves were soon universally adopted in all makes of the Benedict type of apparatus, including the "Benedict student form."<sup>5</sup>

All the electric air blowers installed in metabolism apparatus have the double action of a centrifugal pump: compression at the outlet and suction at the inlet. Depending on the location of the blower, the mouthpiece may be either in a zone of compression (a) or of suction (b). If in zone (a), inspiration will be assisted and expiration hindered, while in zone (b), inspiration will be hindered and expiration assisted. It is evident that breathing from

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\*Received for publication, May 21, 1934.

6. The positive, or negative, pressure maintained by the air blower throughout the entire circuit invites leaks, especially in the region of the mouth and the nose.

7. With the type of metabolism apparatus as now in common use, the electric impeller is unnecessary and sometimes a troublesome impediment.

8. The possibility of asphyxia which may be insidiously induced by the re-breathing of carbon dioxide freed air is not to be ignored, especially in the use of all motor operated metabolism, oxygen therapy, and artificial respiration apparatus.

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and may complain of difficult breathing or of something else. Tact and good technique will almost invariably take care of such subjects.

The use of the motor-blower rather tends to encourage neglect in the proper care of the apparatus and promotes in the mind of the operator a false sense of security even in the presence of more or less serious ventilation obstructions. In fact, too fine, too much, or impacted soda lime; too much resistance due to too long tubings; kinks or obstructions by accumulation of moisture; an improperly balanced spirometer bell, etc., may all be responsible for a partial or even a complete obstruction somewhere in the ventilation circuit. All this may take place during the test without any immediate apparent sign of trouble, while the motor is humming along as usual, while the spirometer bell is rising and falling without interference and while air continues to be available through the mouthpiece. In the absence of the valves, the air in the entire circuit can move in either direction and air will still be available through the mouthpiece in the presence of even a complete obstruction in the circuit. Under such conditions, more or less rebreathing of the expired air occurs and may be temporarily tolerated by the subject. The results of the test, however, should be questioned, chiefly on account of a possible deficiency in the oxygen supply.

There is an element of danger especially connected with the use of all air blower equipped metabolism and other respiration apparatus of the Closed Circuit type, including the various makes of oxygen therapy tents and artificial respirators.<sup>8</sup> I have previously called attention to the not altogether remote danger of acute anoxemia which is readily and insidiously induced by the rebreathing of air which is more or less completely freed from exhaled carbon dioxide. On account of the absence of an increased  $\text{CO}_2$  concentration in the inspired air the body fails to make, for the time being, any discernable protest against an insidious and quite symptomless deficiency of oxygen until a more or less damaging degree of anoxemia or asphyxiation has been induced. Such an accident is practically impossible with a "valve" metabolism apparatus when the spirometer bell has been properly filled with oxygen before the test.

#### SUMMARY

The conclusions drawn twelve years ago can now be reiterated with greater emphasis:

1. The Benedict-Roth metabolism apparatus lends itself admirably to the adaptation of valves and the discard of an air blower.
2. The use of the apparatus is made available independently of any source of electrical energy.
3. The use of valves, together with a properly balanced spirometer bell, promotes easy and normal breathing and insures basal results.
4. The apparatus is absolutely noiseless and free from the possible transmission of disturbing impulses or "hums."
5. The valves seldom require attention. They will never have a tendency to stick if properly mounted and so adjusted that their "lips" just barely come together.



Hemolytic streptococci were found more frequently associated with infection of the upper respiratory tract in rheumatic persons who had not had their tonsils removed than in those on whom tonsillectomy had been performed.

Green-producing streptococci were present in all the cases and in 99 per cent of the cultures. They predominated in 69 per cent of the cases and in 73 per cent of the cultures.

Indifferent streptococci were present in 96 per cent of the cases and in 88 per cent of the cultures. They predominated in 3 per cent of the cultures.

In the groups studied, exacerbations of acute rheumatic fever occurred as frequently when there was no infection of the throat as when there was.

The fact that the majority of those in whom exacerbations of rheumatic fever occurred were persons who had hemolytic streptococci in their throats suggests a possible relationship between this organism and the reappearance of the symptoms.

#### **TETANUS, Acute, Study of the Treatment of, Taylor, F. W. J. A. M. A. 102: 895. 1934.**

1. *Local Lesion:* Since the local wound causes the disease and continues it, prime consideration should be given to that focus. Whenever possible, immediate complete excision of the wound is recommended. When this is not possible, complete exposure and search for foreign bodies should be done under general anesthesia. The foreign body is a potent factor aiding bacterial growth and continuing the elaboration of toxin. It should not be overlooked. In active tetanus cases it must be considered that the local wound contains a foreign body until proved otherwise. This is particularly true of "blank" cartridge wounds. The local wound of tetanus is an acute surgical emergency and should be treated as such, even though the patient is later admitted to the medical wards.

2. *Sedatives and General Care:* Patients with tetanus die from "their symptoms and not from the disease itself." It therefore follows that if these symptoms can be controlled adequately the patient's chance of recovery is much greater. Tribromethanol or sodium amytal is recommended to induce light narcosis. The suggestion is also made that these drugs be given at regular intervals so as to keep the patient quiet and relaxed. This would seem wiser than to use them only when the patient becomes rigid and is on the verge of a convulsion. Equally important in conserving the patient's strength with sedatives is adequate supportive care. Particular attention must be given so that fluid intake is maintained at a high level and proper elimination is effected.

3. *Tetanus Antitoxin Serum:* The high reputation that this product enjoys is due entirely to its prophylactic use. Here it is a specific in preventing the disease or at least in lengthening the incubation period. In active tetanus it is in no sense a specific, and its value is questioned by many. As the antitoxin is unable to withdraw toxin already combined with nervous tissue, its sole action is to neutralize uncombined toxin in the body. Here it may have considerable value and, until definite proof to the contrary, its use may well be continued. It seems unnecessary to administer the huge quantities used at present. It is recommended that from 30,000 to 60,000 units be given when the patient is first seen. This might be repeated if the case runs a long, protracted course. The intramuscular route of injection is perhaps the most satisfactory.

The conclusion of two workers in this field nearly forty years ago is as pertinent today as it was then: "An ounce of clean surgery is worth several pounds of serum therapy."

#### **SYPHILIS AND PREGNANCY, Mc Kelvey, J. L., and Turner, T. B. J. A. M. A. 102: 503, 1934.**

The Wasserman test on the blood of the umbilical cord and study of the placental histology are important aids in the diagnosis of congenital syphilis and should be carried out on all patients not proved during pregnancy to be free from syphilis. Of the two, the former is the more reliable.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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**CHOLESTEROL**, Relation of Plasma to Obesity and to Some of the Complicating Degenerative Diseases, Bruger, M., and Poindexter, C. A. Arch. Int. Med. 53: 423, 1934.

The plasma cholesterol in fifty-three obese subjects, in whom evidence of metabolic, arthritic or endocrine disturbances was lacking, was usually within normal limits.

The advent of diabetes mellitus, essential hypertension or arteriosclerosis in eighteen obese persons was associated in many instances with an augmented cholesterol content of the plasma. Four of seven overweight subjects who demonstrated a diminished tolerance to ingested carbohydrate as determined by the dextrose tolerance test, even though signs and symptoms of diabetes mellitus were lacking, showed an increased plasma cholesterol.

In four obese persons with infectious (rheumatoid or gonococcal) arthritis, the plasma cholesterol was usually within the normal limits; in seven cases with degenerative hypertrophic arthritis or osteoarthritis, the cholesterol was often significantly elevated.

From these observations it is concluded that:

A high calorie diet, presumably high in carbohydrates and fats, which results in obesity is not accompanied by a rise of the plasma cholesterol in human beings.

The development of degenerative diseases (hypertension, diabetes, arthritis and arteriosclerosis) in the obese is as a rule followed, and not preceded, by hypercholesteremia. Therefore, the elevated concentration of the cholesterol in the blood in these degenerative conditions is usually to be regarded as a complication and not in the light of an etiologic factor.

**RHEUMATIC FEVER**, Bacteriologic Study of Throats in Rheumatic and Non-Rheumatic Fever, Weinstein, I., and Styron, N. C. Arch. Int. Med. 53: 453, 1934.

The present investigation includes 321 cases and 840 cultures from Montefiore and Bellevue Hospitals, New York City. Forty-six per cent of the subjects were patients with rheumatic fever. Fifty-eight per cent of the cultures were taken from this group. The remainder were taken from normal persons and from patients suffering from diseases other than rheumatic fever; these constituted the control group. In more than one-third of the cases of rheumatic fever and in more than one-half of the cultures taken from this group the patients were under fifteen years of age.

In making cultures of hemolytic streptococci, it seems that rabbit blood is slightly better than horse blood. However, if appropriate dilutions are made, equally good results can be obtained with horse blood.

The percentage of cultures which were positive for the hemolytic streptococcus taken from the throats of patients with rheumatic fever was approximately the same as that found in other persons.

In these positive cultures, the hemolytic streptococcus appeared to be no more abundant in those from patients with rheumatic fever than in those from other persons.

Throat cultures of patients with rheumatic fever taken during an infection of the upper respiratory tract showed no greater incidence of hemolytic streptococci than those from other persons who were suffering from a cold or from sore throat.

Infections of the upper respiratory tract were found associated with *Streptococcus hemolyticus* more frequently in young persons who were suffering from rheumatic fever than in older patients with this disease.

The total cell counts in 20 children with encephalitis (13 recoveries and 7 deaths) varied from 1 to 110, with an average of 23 cells per cubic millimeter.

In tuberculous meningitis (34 adults and 98 children) the average total cell counts were higher than those for encephalitis in both adults and children.

The average polynuclears and lymphocytes were practically the same in both diseases, but there was always a marked preponderance of lymphocytes over polynuclears.

No definite relation was found between the total cell count in and the severity of encephalitis, even during the different periods of the disease.

As all the tuberculous meningitis cases in our series died, the relation mentioned in the preceding statement could not be determined.

Normal cell counts were found in cases of encephalitis and tuberculous meningitis, and the probable explanations are given.

In a few cases of encephalitis and tuberculous meningitis, especially at the beginning, predominance of polynuclears was observed, which may cause it to be confused with suppurative meningitis; but confusion can be definitely eliminated by further examination of the spinal fluid at different periods.

Finally, in the author's opinion, one cannot rely alone on the cellular counts in the spinal fluid in differentiating between encephalitis and tuberculous meningitis; therefore, we must look for other reliable and quick methods, either in the clinics or in the laboratory.

**KALA-AZAR, New Diagnostic Test for, Nattan-Larrier, L., and Grimard-Richard, L. C. R. Soc. Biol. 113: 1489, 1934.**

The authors describe a new serological test for leishmania infections based on the fact that alexine is adsorbed by a mixture of the serum from a case of leishmania infection and the serum of a rabbit which has been immunized with cultures of *Leishmania donovani*. The rabbit serum is prepared by injecting a rabbit intravenously with a culture every six days until 6 injections have been given. The blood is abstracted 15 days after the last injection, defibrinated and centrifuged, the resulting serum being placed in ampules which are kept in the ice chest. The test is carried out in two ways. In the first 0.1 c.c. of alexine, 0.1 c.c. of the suspected inactivated leishmania serum, decreasing quantities (one-tenth to one hundred millionth of 1 c.c.) of the rabbit serum (inactivated if fresh) and 0.2 c.c. of physiological saline solution are mixed in tubes and kept at a temperature of 37° C. for one and one-half hours. To each tube are then added 0.1 c.c. of antishoop serum and 0.1 c.c. of 5 per cent suspension of sheep corpuscles. The tubes are then kept at 37° C. for one-half hour. If the suspected serum was actually from a case of leishmania infection hemolysis does not occur owing to fixation of the alexine. In the second way of making the test the suspected serum is present in decreasing quantities, the other ingredients being uniform.

The two methods give good results but the second is preferable if small quantities only of suspected serum are available. Positive results have been obtained with one human and six canine cases of kala-azar and with mice suffering from *Leishmania tropica* infection of the tail.

**TYPHOID BACILLUS, A Study of "Smooth" and "Rough" Forms of, In Relation to Prophylactic Vaccination and Immunity in Typhoid Fever, Maltaner, F. J. Immunol. 26: 161, 1934.**

Methods of dissociation were applied to a group of strains of the typhoid bacillus, including recently isolated cultures from "carriers" and several well-known laboratory strains.

Typical smooth and rough variants were obtained from both flagellated and non-flagellated forms. Capsules were demonstrated on all forms: rough, smooth, flagellated, and non-flagellated.

No serologic relationship was observed in agglutination and agglutinin-absorption experiments between the somatic antigens of smooth and rough races of *B. typhosus* although the existence of a common flagellar antigen was confirmed.

The presence of characteristic changes in the epiphyses of the long bones during the first two weeks of life is diagnostic of congenital syphilis, but the absence of epiphyseal abnormalities does not rule out congenital infection.

Antenatal treatment of pregnant syphilitic patients with arsphenamine reduces the percentage of fetal deaths and the percentage of syphilitic infants in a striking manner. The good results are roughly proportional to the amount of treatment given and the time at which it is started; even a few treatments in the last weeks of pregnancy, however, will materially alter the outcome.

**IMPETIGO CONTAGIOSA**, Prophylaxis of, Guy, W. H., and Jacob, F. M. J. A. M. A. 102: 840, 1934.

The following method, used since 1930, has been found efficacious.

*A. Delivery Room.*—

1. As soon as possible after birth, babies are cleansed thoroughly with sterile liquid petrolatum.

2. Each baby is thoroughly rubbed from the top of the head to the soles of the feet with 2 per cent ointment of ammoniated mercury before it leaves the delivery room.

*B. Nursery.*—

1. Daily cleansing is accomplished with sterile cottonseed oil.

2. The buttocks and anus are cleansed with cotton balls and cottonseed oil.

3. Soap and water and powder are not used.

**HYPOCALCEMIA AND MIGRAINE**, Norman, G. F. J. A. M. A. 102: 529, 1934.

A number of cases of typical so-called idiopathic migraine have been studied with respect to blood calcium values, which have been found to be uniformly somewhat depressed. Six cases have been chosen at random, ranging from relatively mild to very severe, the last being associated with epileptiform convulsions. This entire group has been benefited by means taken to raise the values of calcium to normal.

It is assumed that symptoms of migraine and tetany are maintained dormant in this group by the calcium regulating mechanism.

One or the other complex may predominate. Whether unilateral headache and nausea with or without vomiting should be included in the picture recognized as tetany remains for further study.

**MERCURY POISONING**, An Antidote for, Rosenthal, S. J. A. M. A. 102: 1273, 1934.

Sodium formaldehyde sulfoxylate saved nine of twelve dogs from a fatal oral dose of corrosive mercuric chloride, when administered by mouth and intravenously within an hour and a half after the poison had been taken. The nine surviving animals were protected against kidney damage, as shown by the lack of elevation of the blood nonprotein nitrogen. In the dogs that succumbed following this therapy or following intravenous therapy only, no significant renal lesions were demonstrable histologically.

The sulfoxylate was used in ten human cases of acute poisoning from corrosive mercuric chloride, and recovery occurred without appreciable kidney damage.

**SPINAL FLUID**, Cell Counts in Epidemic Encephalitis and Tuberculous Meningitis, Guerrero, L., and Ignacio, P. J. Philippine Islands M. A. 14: 81, 1934.

The total cellular count of the spinal fluids in adult encephalitis cases, numbering 49 (44 recoveries and 5 deaths) varied from 1 to 3,067 with an average of 139. As it is unusual to find epidemic encephalitis with total cell counts amounting to several hundred or thousand cells per cubic millimeter, it is possible that these cases might have been cases of so-called lymphocytic meningitis, the exact cause of which is not very well known.

Of the 14 patients who failed to demonstrate a negative reaction to the Schick test after vaccination, 13 were immunized during the first half year of life. Of the 11 immunized infants whose reactions reverted to positivo, 10 were immunized when under six months of age.

In a small group of infants in whom the duration of immunity could be tested, it was noted that the immunity seemed more lasting in the infants who demonstrated immunity to the Schick test most rapidly.

There is a definite relationship between the antigenic power of the immunizing agent used and the results of vaccination.

Immunization should never be considered complete without a follow-up Schick test.

**ANTIGEN, Method of Diluting, in Relation to Wassermann Reaction, Kolmer, J. A., and Richter, C. E.** *Am. J. Clin. Path.* 4: 301, 1934.

The manner or method of diluting extract for the Wassermann test has a slight but definite influence upon antigenic sensitiveness.

Turbid emulsions of antigens secured by slow or gradual dilution with saline solution are more antigenic than opalescent emulsions prepared by rapid dilution.

Turbid emulsions of antigens prepared by adding extract drop by drop to saline solution, with constant shaking, are more antigenic than turbid emulsions prepared by adding small amounts of saline solution to antigen with constant shaking.

There is no detectable influence in the manner of diluting Kolmer antigen upon the hemolytic properties of this extract.

Turbid emulsions of antigens prepared by slow dilution are sometimes slightly more anti-complementary than opalescent emulsions prepared by rapid dilution.

In the Kolmer modification of the Wassermann test, it is recommended that the antigen be diluted by adding it drop by drop to the required amount of saline solution with constant shaking to secure the maximum of turbidity as originally described in this method.

**"CARRIERS," Practical Limitations in the Attempt to Control Enteric Disease by the Examination of Specimens Collected Without Regard to Clinical History or Epidemiological Evidence, Gilbert, R., and Coleman, M. B.** *Am. J. Pub. Health* 24: 1, 1934.

A false sense of security may result from the examination of a specimen or two submitted from each food handler, since every carrier of *B. typhosus* would not be discovered by this procedure. Even when a number of plates are used in the study and a most careful search is made, *B. typhosus* may be found in only a relatively small percentage of the fecal specimens obtained from some typhoid carriers.

The study of environmental factors, careful history-taking, and the submission of a series of specimens of feces and urine, or preferably a specimen of duodenal contents from food handlers who have had enteric disease, colitis, cholecystitis, etc., or whose immediate associates have had typhoid or paratyphoid fever, would probably result in the detection of most carriers of typhoid or paratyphoid bacilli among them. Of course, there are exceptions. When cases of enteric disease have occurred among patrons of a restaurant or on a milk route, series of specimens from all the food handlers concerned should be examined if the carrier is not found among those with suggestive histories.

Despite the fact that unrecognized cases occur, probably one of the best means of approaching the problem is by studying specimens from patients who are convalescing from typhoid fever or allied infections, in order to detect the ones who will become carriers. Since the submission of specimens from persons who have recovered from typhoid fever has been required by the New York State Sanitary Code, no cases of the disease, as far as the authors are aware, have been traced to an individual who has been released after fulfillment of the requirements.

In order to avoid the fruitless expenditure of time and materials in the examination of large numbers of specimens that have been collected without discrimination from various groups of individuals, the relative futility and excessive cost of such work should be ex-

Living suspensions of all flagellated and of some nonflagellated rough forms failed to be agglutinated by pure rough somatic antiserum. They did agglutinate, however, when the suspensions were heated to a temperature which destroys flagella and probably also capsules.

By intravenous inoculation of rabbits with suspensions of living rough and smooth variants, the relative invasive and toxic character of these forms was determined. No differences in toxicity were observed by this method. Only the smooth variants, however, were found to possess invasive properties as determined by development of the carrier state.

Vaccination of rabbits by the intravenous method with adequate dosage of either rough or smooth variants gave protection against approximately fourteen times the minimal invasive dose of smooth living bacilli. The rough vaccines protected distinctly better than the smooth. The sera of rabbits vaccinated with nonflagellated rough vaccines did not possess specific agglutinins for the smooth infecting strain. Those vaccinated with rough flagellated forms possessed only flagellar agglutinins.

The inoculation of normal rabbits intravenously with rough variants of the typhoid bacillus resulted in a more marked and persistent leucocytosis than when smooth bacilli were used. Immunized rabbits, regardless of whether rough or smooth vaccines had been used, responded after the usual primary leucopenia with a hyperleucocytosis upon intravenous injection of smooth living cultures, whereas the response of unvaccinated rabbits to the injection of virulent smooth bacilli was relatively slight.

The intraperitoneal inoculation of living rough and smooth typhoid bacilli into guinea pigs indicated that the smooth variant was more toxic than the rough. However, the inoculated smooth bacilli multiplied very rapidly in the peritoneum whereas similar doses of the rough variant were disposed of by prompt phagocytosis.

The rough variant, as indicated, is phagocytosed more vigorously than the smooth in the peritoneal cavity of the guinea pig—both within the peritoneal exudate and on and within the omentum and liver. This phagocytic response to rough organisms in normal guinea pigs parallels the reaction obtained when smooth bacilli are inoculated into the peritoneal cavity of guinea pigs immunized with either rough or smooth vaccines.

The results of this experimental investigation are, in every particular, in conformity with the practical experience of the past thirty years in preventive inoculation of human subjects against typhoid fever.

#### DYSENTERY BACILLI, Classification of, Shiga, K. Zentralbl. Bakt. 130: 1, 1934.

Shiga proposes that the dysentery bacilli be divided into the following groups: (1) dysentery, (2) metadysentery, and (3) paradysentery.

The differentiation is shown in the table:

ACCORDING TO LENTZ	1	2	3			4
	SHIGA-KRUSE BACILLUS	SCHMITZ BACILLUS	FLEXNER GROUP			KRUSE-SONNE BACILLUS
			FLEXNER	Y	STRONG	
Litmus mannite	blue	blue	red	red	red	red
Indole	—	+	+	±	±	+
Milk	no coagulation	no coagulation	no coagulation			coagulation
Litmus lactose	blue	blue	blue			red
Special toxin	+	—	—	—	—	—
According to Shiga	(1) <i>B. dysen- teriae</i>	(2) <i>Bacillus metadysenteriae</i>			(3) <i>B. para- dysenteriae</i>	

#### DIPHTHERIA TOXOID, Immunity Produced by, Greengard, J. Am. J. Dis. Child. 47: 799, 1934.

In a series of 214 susceptible infants ranging from newborn to two years of age, 93.4 per cent showed a negative reaction to the Schick test after two injections of 1 c.c. of commercial diphtheria toxoid.

Of 63 immunized infants retested at intervals after the first negative reaction to the Schick test, 11 or 17.2 per cent reverted to a positive reaction.

# The Journal of Laboratory and Clinical Medicine

VOL. 20

JANUARY, 1935

No. 4

Editor: WARREN T. VAUGHAN, M.D.

Richmond, Va.

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## EDITORIAL

### The Problem of Peptic Ulcer

IN LAST analysis, the etiology of peptic ulcer still remains an unsolved problem. It seems probable that of the many etiologic factors that have been suggested in the past, several or all may play a part at times, but no one explanation or group of explanations so far proposed seems to present a complete solution in all cases. Metastasis from foci of infection, corrosive action of hydrochloric acid, enteric excretion of toxic substances, trauma, and excess heat may be factors of importance in individual cases but there is also evidence of a fundamental predisposition, in the absence of which these factors remain inactive.

The problem of trauma is indeed interesting. One of the difficulties of experimental investigations on the etiology of ulcer appears to be the great difficulty in producing chronic ulcer in animals. Traumatic ecchymoses may be produced in the stomach wall hematomas, and indeed these may become infected, resulting in acute ulceration. But such an experimentally produced

plained to members of county medical societies and health officials. The same amount of money would yield vastly greater returns if expended in the pasteurization of products wherever possible, the improvement of sanitary facilities, the provision of training in personal hygiene, the removal from work and the treatment of food handlers who are obviously ill, the careful study of epidemiological factors, and the examination of series of specimens from individuals whose history or clinical manifestations warrant it.

**PSITTACOSIS, Pathology of, Foord, A. G. Am. J. Clin. Path. 4: 247, 1934.**

The principal finding in fatal human cases of psittacosis is a unique pneumonia, involving diffusely the greater part of all of one or more lobes, producing a wet, consolidated parenchyma which is smooth and nongranular on section. The pleura, bronchi, and bronchioles are relatively spared. Histologically the consolidation is found to be due to an exudate consisting of fluid and varying amounts of fibrin, in the meshes of which large numbers of large mononuclear cells are found. Proliferation of the alveolar epithelium and histiocytes in the alveolar walls is a constant finding.

Moderate splenic tumor and congestion and cloudy swelling of the parenchymatous viscera is the rule.

Edema and congestion of the brain and leptomeninges is constant, ring-shaped hemorrhages in the cord and brain have been found in some cases, but thorough neurologic study should be made on all cases, since delirium and psychosis are clinically found in the majority of cases.

Postmortem diagnosis should be confirmed by inoculating white mice with material from the lung, liver, and spleen. Antemortem diagnosis is usually not possible before the patient recovers or dies but can sometimes be made by inoculation of a series of white mice intraperitoneally with the patient's blood, or better with the sputum.

**ARTHRITIS, Chronic Nonspecific, Archer, B. H. J. A. M. A. 102: 1449, 1934.**

There appears to be a basis for the concept that both rheumatoid arthritis and osteoarthritis are due to the same etiologic agent or group of agents and that the proliferative and degenerative pathologic changes by which the two types manifest themselves are the result of other factors than those of causation.

There seems to be no conclusive evidence of the presence of streptococci in the blood and joints of patients with chronic arthritis.

None of the vaccines employed at the present time in the treatment of chronic arthritis have been accepted by the Council on Pharmacy and Chemistry. There is no evidence at hand that they exercise any specific effect on the course of the disease.

Dietary regulations and vitamin therapy apparently exercise no specific effect on the joint manifestations of patients with this disease.

In those cases associated with foci of infection it seems wise to search for and remove this factor early in the course of the disease.

In advanced cases the measures the author has found to be of greatest benefit to the patient are orthopedic procedures, physical therapy, the administration of drugs to allay pain, and a change of climate.

Present knowledge of the subject does not seem to warrant the view that certain definite measures should be applied only to certain definite types of arthritis. There is no conclusive evidence that the same measures do not apply at some time to all forms of non-specific arthritis.



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T. B. MAGATH, M.D.	- - - - -	ROCHESTER, MINN.
DEAN LEWIS, M.D.	- - - - -	BALTIMORE
M. H. SOULE, Sc.D.	- - - - -	ANN ARBOR, MICH.
CLIFFORD J. BARBORKA, M.D.	- - - - -	CHICAGO

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## EDITORIAL

### The Problem of Peptic Ulcer

IN LAST analysis, the etiology of peptic ulcer still remains an unsolved problem. It seems probable that of the many etiologic factors that have been suggested in the past, several or all may play a part at times, but no one explanation or group of explanations so far proposed seems to present a complete solution in all cases. Metastasis from foci of infection, corrosive action of hydrochloric acid, enteric excretion of toxic substances, trauma, and excess heat may be factors of importance in individual cases but there is also evidence of a fundamental predisposition, in the absence of which these factors remain inactive.

The problem of trauma is indeed interesting. One of the difficulties of experimental investigations on the etiology of ulcer appears to be the great difficulty in producing chronic ulcer in animals. Traumatic ecchymoses may be produced in the stomach wall hematomas, and indeed these may become infected, resulting in acute ulceration. But such an experimentally produced

## CORRESPONDENCE

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To the Editor:

Dear Sir: The purpose of this communication is to call attention to the fact that we have been misquoted in the article by L. F. Herz (*JOURNAL OF LABORATORY AND CLINICAL MEDICINE* 20: 33, 1934), in which he states on page 39, "Since this article was written Kracke and Parker have reported several cases following the use of 'empirin,' a mixture of phenacetine, aspirin and caffeine." First, we did not report several cases following the use of this mixture, and second, empirin is not a mixture of phenacetine, aspirin, and caffeine; but only a trade name for acetylsalicylic acid. Further comment relative to this article seems indicated.

First, it is evident that the author has attempted to show that the leucocyte depressing effect of amidopyrine is due to its pyrazolon attachment. We do not believe that he establishes this. He further states, "I am prepared to show that amidopyrine should not be classed as a benzene ring derivative." We do not believe that he has demonstrated this. After close examination of the chemical reactions presented by the author we note many errors; for example, McGuigan states that amidopyrine is a derivative of phenylhydrazine that comes ultimately from aniline and benzene and is stated by that author to be a benzene derivative. It is not prepared from pyrazolon.

It is stated on page 36, that amidopyrine is more toxic than acetanilid and phenacetine, not because of the pyrrol ring, but because of the NH component of the ring. This NH or N is called, on page 37, an imido radical and to this fact is assigned its toxicity. It must be pointed out that this structure is not an imido radical but an amine radical and occurs, not only in amidopyrine but also in acetanilid and phenacetine. An imido radical must be attached to two acyl groups, otherwise it is an amine as in the case of amidopyrine.

On page 37, is shown by diagram, several drugs which are said to have the NH or N group as an integral part of the ring. In this group are listed both anilin and phenyl hydrazine, neither of which has these groups in the ring. Instead they are merely side chains attached to the ring.

On the same page it is stated that piperidine differs from pyridine only in the substitution of an NH radical for the N radical. This is in error since pyridine has a constitutional formula of  $C_5H_5N$  while piperidine has the formula  $C_4H_{11}N$ .

It is also stated, page 37, that phenyl hydrazine contains the imido radical, which is in error as this NH is an amine.

Since the author presents little evidence to show that amidopyrine is not a benzene ring derivative, it follows that some of his conclusions are unwarranted. Although there is much evidence to indicate that amidopyrine is a dangerous leukocyte depressing drug only in an occasional person; its dangerous character has not been so well established that it should be banned by the medical profession. Amidopyrine is a very valuable drug when properly used and in all probability it should continue to be used with caution just as we continue to use neoarsphenamine, although we recognize its ill effects in an occasional person.

The author goes beyond the scope of his article in drawing conclusion number six when he states that acetanilid is the drug of choice when anodynes or antipyretics are indicated. And still further unwarranted is the conclusion that it should be mixed with caffeine and potassium citrate, since there is no scientific evidence to show the desirability of using such a mixture in therapeutics.

ROY R. KRACKE, M.D.,  
AND  
FRANCIS P. PARKER, M.D.

NOVEMBER 5, 1934,  
EMORY UNIVERSITY, GA.

# *The Journal of Laboratory and Clinical Medicine*

VOL. 20

FEBRUARY, 1935

No. 5

## CLINICAL AND EXPERIMENTAL

### DIPHTHERIA IMMUNIZATION WITH A SINGLE INJECTION OF HIGHLY PURIFIED FORMOL-TOXOID AND AL(OH)<sub>3</sub>\*

CHARLES N. LEACH, M.D., NEW YORK, N. Y.,  
CLAUS JENSEN, M.D., COPENHAGEN, DENMARK,  
AND GEORG PÖCH, M.D., EISENSTADT, AUSTRIA

FORMOL-TOXOID (anatoxin) introduced by Ramon as an agent of active immunization against diphtheria is rapidly replacing all other diphtheria prophylactics. In some countries mass immunization by the usual three-injection method has been possible. In France over one million and in Hungary more than half a million children have been so immunized. The formol-toxoid has been employed without mishap, and experience hitherto obtained seems to indicate that from 90 to 95 per cent of persons receiving three injections are immunized against diphtheria within six to seven weeks after the first injection. This naturally assumes that the toxoid is sufficiently high in antigenic value.

The three-injection method has always been subject to more or less opposition on the part of the general public. This is brought out in some recent publications by the fact that the number of children receiving the second and third injection is considerably smaller than the number enumerated under the first injection. The most significant drop generally occurs after the second

\*From the International Health Division of The Rockefeller Foundation; State Serum Institute, Denmark; and the Local Health Department, Eisenstadt, Austria.  
Received for publication, June 6, 1934.

The studies and observations herein reported were conducted with the support and under the auspices of the International Health Division of The Rockefeller Foundation. The investigation was made possible through the consent and approval of the Volksgesundheitsamt of the Austrian Ministry of Social Welfare.

Serological work was done by the State Serum Institute, Copenhagen, Denmark, of which Dr. Thorvald Madsen, is director.

injection, as this is most frequently followed by local or general reactions (Claus Jensen, 1931<sup>1</sup>). The general effectiveness of the three-injection method is thus reduced, and the percentage of immunized in a mass campaign may be only 80 per cent or less. This fact should be borne in mind when drawing comparisons between the value of the three-injection method as opposed to others.

It would therefore be of significance to produce, if possible, a diphtheria antigen of such marked immunizing power that, given in a single subcutaneous injection, it would produce a suitable degree of immunity without causing undue local or general reactions.

Using a method devised by Willstätter for the purification of enzymes, S. Schmidt, A. Hansen, and K. A. Kjaer (1931) of the Copenhagen State Serum Institute succeeded in producing purified, concentrated diphtheria formol-toxoids containing high amounts of antigen per cubic centimeter, which were innocuous when injected into guinea pigs in 5 c.c. amounts. Furthermore, in experiments with such preparations in horses and guinea pigs, an immunizing power was demonstrated equal to or even higher than that obtained with the corresponding crude toxoids (S. Schmidt, 1931). A single injection of these purified toxoids protected guinea pigs against ten lethal doses of diphtheria toxin given three weeks later. Consequently the above-mentioned authors suggested that such preparations might be used advantageously for immunization of human beings.

The effects of these preparations, when injected into children, have been investigated by Claus Jensen (1931,<sup>2</sup> 1933<sup>1</sup>). He demonstrated that a single injection of these toxoids gave rise to a production of antitoxin which in some cases was rapid and considerable, without causing more pronounced reactions than the first injection of ordinary formol-toxoid. Further investigation (see Claus Jensen, 1933,<sup>2</sup> §10) showed, however, that the crude formol-toxoids varied considerably in immunizing power. These variations were also found after purification, but no alteration of the specific properties occurred during the purification process by the method mentioned above. In many instances these variations did not depend upon the flocculation value as measured by Ramon. While some preparations, in a single dose of 200 to 300 flocculation units, would give a satisfactory result (92 per cent Schick negative within twenty-eight days), others with a flocculation value as high as 1,000 units per c.c. would not produce a like effect. Until a satisfactory method can be developed for determining these qualitative differences in the various preparations, it is difficult to settle the question of dosage.

Another important point is that the purified toxoids are possibly eliminated more rapidly from the body, as the experiments of Glenny, Buttle, and Stevens (1931) seem to indicate.

Roux and Yersin (1889) demonstrated that metallic salts when added to diphtheria broth form precipitates which retain smaller or larger amounts of the antigen. Later the effect of several of these nonspecific substances was investigated: among them were tapioca (Ramon); alum (Glenny, Pope, Waddington, and Wallace, 1926; Glenny, 1931; Glenny and Barr, 1931; Llewellyn

Smith, 1932); calcium (Ramon and Nélis, 1931); and others. It was found that these additions caused a considerable increase in the antigenic efficiency of the toxoid. This effect is probably due to some fixation of the antigen at the site of injection, causing a slower absorption and, therefore, a more prolonged stimulus. Unfortunately, adding these substances to the ordinary toxoid in most instances causes rather severe local reactions when the preparation is injected into man. The admixture of alum has been used by Park in the United States with good results. The toxoid contained, however, only small amounts of alum (0.2 per cent), and the usual three injections were used by Park. Larger amounts of alum (2 per cent) were used in guinea pigs in a single injection by Pondmann and Tasman (1931) but caused extensive hard infiltrations of the tissues of long duration at the site of injection. Such infiltrations may give rise to abscess formation, even a considerable time after injection. Thus Saunders (1933) observed four cases of abscess formation in children; one of these cases occurred three weeks after and another over one year after the alum-toxoid injection (7.5 per cent alum).

These by-effects naturally reduce the usefulness of such material when intended for mass immunization of children.

More promising results have been obtained by S. Schmidt (1932), who in place of alum has used the Willstätter preparation of alum hydroxide  $Al(OH)_3$ . This preparation has a high specific combining capacity for purified toxoid. A small quantity might therefore be sufficient to obtain the desired effect. The method of preparation of this antigen and the results of animal experiments have been published by Schmidt and Hansen (1933).

This toxoid  $Al(OH)_3$  preparation was first used in a single injection as an immunizing agent on Danish children by Claus Jensen, S. Ahrend Larsen, and K. Bojlén. Approximately 6,000 children have been immunized in this manner. A report of the study will be published later. The present paper records the results obtained with the same preparation in immunizing children in the Bezirk Mattersburg, Austria.

Mattersburg, capital of the county by the same name, is situated in the Province of Burgenland, which partially forms the boundary between Austria and Hungary. It is a town of 3,706 inhabitants, and although it lies in the heart of a farming area the townspeople live for the most part under crowded conditions. The undue prevalence of diphtheria during the past few years has resulted in a heavy financial burden on the community and has made it easy to persuade the parents to present their children for immunization. The incidence of the disease during the six-year period 1928-33 was as follows:

YEAR	DIPHTHERIA CASES
1928	12
1929	5
1930	10
1931	138
1932	93
1933	38

Children of Mattersburg in the age group from two to eleven years inclusive were selected for the immunization campaign. This group consisted of 728 children, of whom 553 were brought to the dispensary for treatment.

A record card was made out for each child. These cards, with the relevant data recorded thereon, are on file at the County Health Department. This material will furnish a basis for a future study of the progress of the disease.

A highly purified formol-toxoid, Al/31, 25 Fl. units per c.c. with 10 per cent by volume of Al(OH)<sub>3</sub>, was supplied by the Copenhagen State Serum Institute.

The toxoid was administered on Nov. 21, 1933, in 2 c.c. doses, injected subcutaneously at the inferior angle of the left scapula. Of the 553 children receiving a single injection, 71 (12.8 per cent) showed local or general reactions. These reactions are generally supposed to result from a hypersensitivity to the specific diphtheria antigen. Even with a highly purified toxoid such as was used here, one might therefore find a higher percentage of reactions among children with a previous history of diphtheria.

TABLE I

REACTIONS OF 123 CHILDREN WHO HAD HAD DIPHTHERIA AND 430 CHILDREN WITH NO HISTORY OF DIPHTHERIA, TO FORMOL-TOXOID IN 2 C.C. DOSES INJECTED SUBCUTANEOUSLY

	CHILDREN WHO HAD HAD DIPHTHERIA (123)		CHILDREN WITH NO HISTORY OF DIPHTHERIA (430)	
	NO.	PER CENT	NO.	PER CENT
Swelling	3	2.4 ± 0.94	6	1.4 ± 0.38
Swelling and redness	24	19.5 ± 2.41	38	8.8 ± 0.92
Temperature above 38° C.	16	13.0 ± 2.05	25	5.8 ± 0.76
Total reactions	43	35.0 ± 2.90	69	16.0 ± 1.20

As Table I indicates, the reactions in the group of children who had had diphtheria were twice as frequent as in the group with no history of diphtheria, thus supporting the above view. This might also explain the fact that the number of reactions observed is distinctly higher than that found with the same preparation in Denmark. It should finally be stated that in no child were the reactions alarming in character. There were no abscess formations in this group.

#### STUDIES OF THE EFFECTIVENESS OF THE IMMUNIZING PREPARATION USED

Instead of employing the Schick test, the reliability of which will be treated in a subsequent paper, the antitoxin content of the serum was accurately determined in blood samples taken immediately before and twenty-eight days after the injection of antigen. This time interval was chosen in order to make the Mattersburg results comparable to those previously obtained in Copenhagen with the same antigen.

The antitoxin determinations were made at the State Serum Institute in Copenhagen by the intracutaneous rabbit method of Claus Jensen (1933<sup>2</sup>), and were not carried out in the usual way, that is, by testing the serum for a value above or below a certain quantity of antitoxin, i.e., 0.03 or 0.01. On the contrary, every blood sample was accurately titrated by two or three

TABLE II  
 ANTITOXIN TITER, PRIOR TO FORMOL-TOXOID ADMINISTRATION, OF 181 CHILDREN WHO HAD NOT HAD DIPHTHERIA

ANTITOXIN TITRE, 1:8000													
	AGE												TOTAL
	2	3	4	5	6	7	8	9	10	11	12	15	
0.0001						2	1		1				6
0.00032						3	9	10	13				57
0.001	1	3	6	2	5	8	6	6	5				24
0.0032			1	3	2	5	2	7	4				44
0.01			4	6	4	13	1	1	3	1	1		10
0.032				1	1	2	2	3	4				14
0.1				1	1	2	4	3	3				14
0.32					2	1		1	2				6
1.0							2		1			1	3
3.16													3
10.0													
31.6													
Total	1	3	11	13	19	36	27	32	36	1	1	1	181
Log. mean	0.00055	0.00055	0.0014	0.0039	0.0068	0.0044	0.0072	0.0058	0.0055	0.018	0.055	18.0	0.0061

successive determinations of increasing exactitude. In this manner each titration served as a control for the preceding titration on the same serum and any chance of error was thus avoided. The method makes possible the detection of traces of antitoxin as low as 0.00005 units per c.c. of serum. Owing

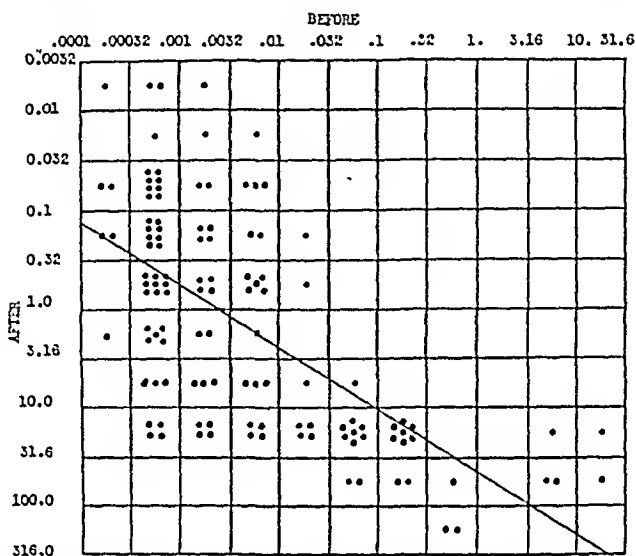


Fig. 1.—Correlation between serum antitoxin content before and after formol-toxoid administration in 120 children who had not had diphtheria.  $r = +0.618 \pm 0.036$ . Mean titer before toxoid 0.00160, mean titer after toxoid 1.43, standard variation before toxoid 13.5%, standard variation after toxoid 13.1%.

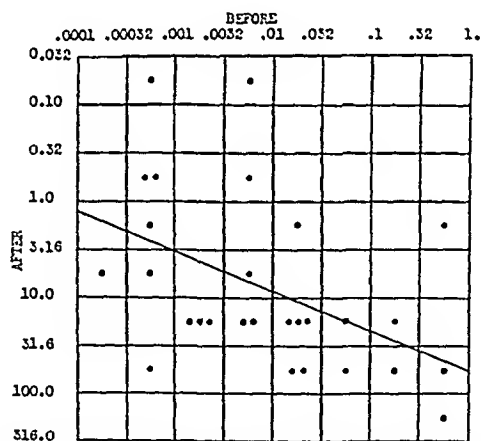


Fig. 2.—Correlation between serum antitoxin content before and after formol-toxoid administration in 28 children who had had diphtheria.  $r = 0.455 \pm 0.105$ . Mean titer before toxoid 0.00349, mean titer after toxoid 7.81, standard variation before toxoid 10.0%, standard variation after toxoid 7.7%.

to insufficient serum it was not possible to test for values below 0.0005 units per c.c. of serum.

We wish to emphasize that the above-mentioned blood sampling at the time of immunization and at a definite period thereafter, in order to determine the antitoxin content, is the most exact means of establishing the value of the antigen and of selecting the immunization method to be employed



in diphtheria prophylaxis. It is needless to say that this procedure is not practicable in mass immunization.

Table II gives the preliminary antitoxin titer, by ages, for 181 children of Mattersburg who had not previously had diphtheria, and Table III gives this titer for 87 children who had had diphtheria. Figs. 1 and 2 show the correlation between serum antitoxin content before and after the administration of formol-toxoid in 120 children who had not had diphtheria (Fig. 1) and in 28 children who had had the disease (Fig. 2).

One might expect that the presence of some "natural" antitoxin in the blood of children would indicate that the antitoxin producing mechanism of these individuals is developed and in a more or less responsive state. This assumption is supported by the well-known fact that horses with small amounts of natural antitoxin are generally the most suitable for routine production of antitoxin of high potency (Glenny). Figs. 1 and 2 show that this assumption holds true for children, and they demonstrate the importance of the primary blood titration in comparing results of immunization by the same or different methods in groups of children.

TABLE III

ANTITOXIN TITER, PRIOR TO FORMOL-TOXOID ADMINISTRATION, OF 87 CHILDREN WHO HAD HAD DIPHTHERIA

	AGE								TOTAL
	3	4	5	6	7	8	9	10	
0.0001							1		1
0.00032	1		2	2	3	1	1	.3	13
0.001					1	2	5	2	10
0.0032			1	2	3	3	2	1	12
0.01		1	1	2	1	2	1	2	10
0.032			2	2	1	3	2	1	11
0.1		1				1	4	1	7
0.32			1	1	7	1	4	1	15
1.0							3	2	5
3.16				1			1		2
10.0						1			1
31.6									
Total	1	2	7	10	16	14	24	13	87
Log. mean	0.00056	0.056	0.013	0.022	0.032	0.025	0.046	0.018	0.027

During the five months following administration of antigen seven cases of diphtheria were reported from Mattersburg. All of these cases occurred among the smaller group (175) of unimmunized children.

The town of Mattersburg has experienced an unusually high diphtheria rate for the past three years with a low case fatality rate. The excessive percentage of diphtheria cases may be taken as an indication of a large number of carriers. It has been impossible in connection with this study to undertake a carrier survey in the town. The patients who have recovered have most probably been exposed to frequent contacts with diphtheria bacilli. The disease, according to previous experiences (Rosling, 1928, 1929, 1930), acts only as a weak primary stimulus. In many instances in this special milieu, however, such a weak stimulus appears to have been sufficient to produce a basic

immunity. The frequent secondary stimuli have given rise to the production of "natural" antitoxin, which in some cases must be considered extremely high. Titers of 11.7, 12.4, and 22 were found prior to antigen administration.

It might be mentioned that the "negative" phase feared by many clinicians in direct relation to toxoid administration and the supposed increased susceptibility to diphtheria resulting from this "negative" phase have not been demonstrable in any case. This is significant in view of the fact that the antigen injections were carried out in an area where diphtheria was prevalent.

Certain objections have been advanced with regard to the single injection method of immunization, the principal claim being that the duration of immunity after one injection of antigen should be shorter than after the three-injection method. Claus Jensen (1931,<sup>2</sup> 1933<sup>1</sup>) has carefully studied the antitoxin production curve after both a single injection of purified and concentrated toxoid and the customary three injections. He found that there was no essential difference in the decrease of antitoxin concentration after the peak was reached, whether produced by one injection or more than one. In both cases the regular fall follows closely the equation for the bimolecular reaction, this having been proved to be a universal biologic law applicable to both man and animals.

#### SUMMARY AND CONCLUSIONS

1. This study was carried out with the single injection method of purified formol-toxoid and  $\text{Al}(\text{OH})_3$  in an area where diphtheria was unusually prevalent. Five hundred and fifty-three children were injected, leaving 175 of the same age group (two to eleven years inclusive) as controls.

2. Local or general reactions to the antigen were observed in 12.8 per cent of the children injected. No cases of severe reactions or abscess formations were recorded.

3. The percentage of reactions to antigen injection was twice as high among children with a previous history of diphtheria.

4. The effectiveness of the antigen was studied by serum titrations before injection and again twenty-eight days after the injection in 148 children. In all cases an increase in antitoxin titer was found, and in by far the majority of cases this increase was considerable.

5. No evidence of a negative phase was found.

6. No cases of diphtheria have occurred among the immunized after the immunization, although seven cases were reported in the control group.

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## A CHEMICAL STUDY OF LYMPH IN EXPERIMENTAL PNEUMONIA\*

JOHN STAIGE DAVIS, JR., M.D., NEW YORK CITY, AND A. J. DELARIO, M.D.,  
PATERSON, N. J.

TO UNDERSTAND the pathology of pneumonia, besides the local anatomical lesion and pulmonary function, the general changes in tissue metabolism must be studied. Some of these changes are not specific for pneumonia, and can be attributed to the fever and the increased expenditure of energy. The high protein metabolism is also found in febrile stages of other diseases. The cause of this increased protein breakdown is generally thought to be connected with the direct action of the toxins upon the tissue. There are, however, chemical changes in the body fluids which are more characteristic of pneumonia, such as decreased oxygen saturation of the arterial and venous blood, a retention of chlorides, and an increase in lactic acid, with which we are directly concerned in the present communication.

### ANOXEMIA

It has been shown by Stadie,<sup>1</sup> by Hastings, Binger, Morgan, and Neill,<sup>2</sup> that in severe pneumonia the oxygen content and saturation are often abnormally low in both arterial and venous blood. This is the case especially when the process in the lungs is diffused and circulation continues through the affected parts. The presence of an exudate in the alveoli impedes the absorption of oxygen. If, however, there is such focal consolidation that the blood passes largely through the perfectly aerated portion, only negligible changes are observed in the oxygen saturation of the blood. It has been shown<sup>3</sup> that as much as half the lung volume may be obliterated without causing changes in the oxygen saturation, as long as the circulation is stopped in that part. Shallow breathing in pneumonia occasionally<sup>4</sup> produces an accelerated flow through the affected area,<sup>5</sup> and this, although it plays a minor part, still has an appreciable rôle in the low oxygen saturation of the arterial blood.

In attempting to understand the more intimate state of the tissue metabolism, one must have more than the data on oxygen consumption, that is, on the difference between the arterial and venous oxygen content. In fact Stadie has reported that in a series of 33 cases of pneumonia (chiefly postinfluenzal), the oxygen consumption was in the usual range for the normal individual, averaging from 3 to 5 c.c. per 100 c.c. of blood. The results are interpreted as indicating a normal cardiac output. The fact that oxygen consumption is in the normal

\*From the Department of Experimental Surgery, Cornell University Medical School.

Received for publication, June 13, 1934.

This work was aided in part by a grant from Mrs. John L. Given.

The writers wish to express their indebtedness to Dr. Joshua E. Sweet under whose supervision this work was done.

range in pneumonia does not necessarily indicate that the gaseous exchange and metabolism in the tissues are normal. It is conceivable that the heightened rate of metabolism and the decreased oxygen tension in the tissues, tend to counter-balance and result in fairly normal figures for oxygen differences between arterial and venous blood. That there is not sufficient oxygen to support complete combustion in the tissues is the interpretation which may be placed on the findings of increased lactic acid. An increase in lactic acid in the body fluids is one of the results of oxygen want. Palmer<sup>6</sup> reported an increase in organic acids in the urine of certain patients with lobar pneumonia. Part of this increased excretion of organic acid was identified as lactic acid by Holten.<sup>7</sup> An accumulation of organic acids in the blood was demonstrated by Peters, et al.<sup>8</sup> and Gervell<sup>9</sup>



Fig. 1.—X-ray film of Dog 1, with right lower lobe pneumonia. Notice anoxemia in both blood and lymph.

showed that this was partly due to lactic acid. It was thought that further insight into the question of the extent of oxygen deficiency in the tissues could be gained by studying the lactic acid content of the lymph, since this fluid originates in intimate contact with the cells, and probably often reflects metabolic changes with more sensitivity than does the blood.

Even in those cases of pneumonia which show anoxemia, no  $\text{CO}_2$  retention has been demonstrated in the blood. Binger, Hastings, Sendroy<sup>10</sup> made observations on a group of 30 patients and found that the  $\text{CO}_2$  content varied from 39.3 to 59.7 volumes per cent, with the average of 49.4. The acid base balance,  $\text{CO}_2$  content, and pH, were all found to be normal.

It occurred to us, because of the increased lactic acid formed, that there might be a decreased  $\text{CO}_2$  production by the tissues, thus producing a normal pH,

as has been found. We determined the  $\text{CO}_2$  content of the lymph in dogs with experimental pneumonia. The effect of oxygen therapy on lymph oxygen was also determined.

#### CHLORIDE RETENTION

It has long been known that there is a disturbance in the chloride balance of the acute stages of pneumonia. The concentration of chlorides falls in both serum and urine, with a simultaneous drop in serum base. The conclusion drawn from this is that sodium chloride is stored in tissues in spite of a deficit in the serum. It has been shown<sup>11</sup> that the depletion in serum chlorides cannot be attributed entirely to low diet, oxygen want, surgical shock, leucocytosis, or hyperthermia. The situation has been summed up by Sunderman<sup>12</sup> in the conclusion



Fig. 2.—X-ray film of Dog 2, with right lower lobe pneumonia.

that in the precritical period pneumonia is characterized by a diminished capacity to conserve chlorides when the chloride intake is low, and a diminished capacity to excrete chloride on a high chloride intake. This is not due to deficiency in renal function, which in pneumonia is only slightly impaired, if at all.<sup>13</sup> The apparent storage of chloride on the normal level of intake, cannot be accounted for by the loss of chloride in the sputum or sweat.<sup>12</sup> It still appears uncertain whether the retained chloride is stored in increased concentration in certain tissues or whether the chloride which enters the tissue is merely stored in edema fluid, with the chloride content in approximate equilibrium with that of the blood plasma.

#### EXPERIMENTAL

For these experiments, pneumonia was produced in six dogs by bronchial insufflation of pneumococcus according to the method of Coryllos and Birnbaum.<sup>14</sup> The injections were made by Dr. Birnbaum. Amytal anesthesia was

used. The presence of pneumonia was ascertained by x-ray examination and at autopsy. The x-ray photographs are reproduced here (Figs. 1 to 6). At the height of the disease, the thoracic duct was exposed, a cannula was inserted, and sufficient lymph for study was collected. The cannula was inserted below the



Fig. 3.—X-ray film of Dog 3, with very severe right lower lobe pneumonia. Dog died thirty minutes after experiment was finished. Notice how low the oxygen tension is in both blood and lymph.



Fig. 4.—X-ray film of Dog 4, after right lower lobe pneumonia. The pneumonia is nearly gone, the lymph oxygen is nearly normal, chlorides are increasing, and lactic acid is diminishing.

level at which the pulmonary drainage enters, in order not to confuse any changes taking place in the body as a whole with those confined to the process in the lungs. To prevent clotting of the lymph, a few crystals of potassium oxalate

were inserted into the cannula, and the cannula was filled with oil under which the lymph collected. Samples were obtained when desired by withdrawing lymph

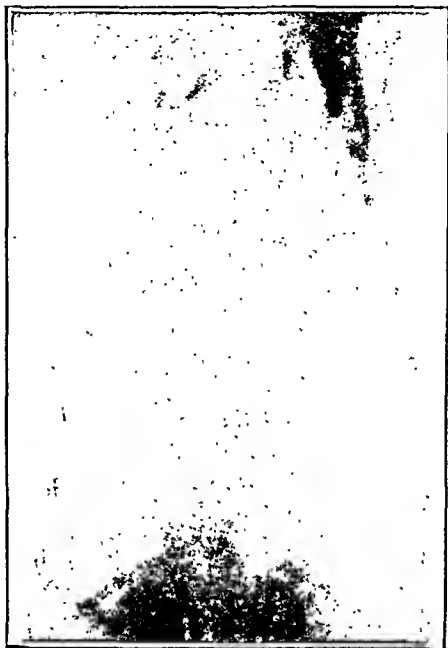


Fig. 5.—X-ray film of Dog 5, with right lower lobe pneumonia.



Fig. 6.—X-ray film of Dog 6, after right lower lobe pneumonia. Pneumonia is nearly gone, dog is not sick, oxygen tension is nearly normal, chlorides are returning to normal. The  $\text{CO}_2$  tension is yet rather low, while lactic acid content is nearly normal.

from below the oil layer by means of a syringe, also containing oil. Blood samples were withdrawn from the femoral vein. So far as possible for the



measurement of  $\text{CO}_2$ , chloride, and lactic acid concentration, the blood and lymph were obtained from these dogs simultaneously.

In three experiments the dogs were fed high fat diets (cream and olive oil) from one to three days before operation, whereas three of the animals had been fasted. The chemical procedures used for the analysis were as follows:  $\text{CO}_2$  by the Van Slyke and Neill method,<sup>15</sup> chloride by the Van Slyke method,<sup>16</sup> and lactic acid by the method of Friedman, Cotonio and Shaffer.<sup>17</sup>

## DISCUSSION OF RESULTS

*Lactic Acid.*—From the results tabulated, it can be seen that the lactic acid in venous blood in animals ill with pneumonia was not definitely increased above the normal (Table I). Examination of the figures for lactic acid concentration in the lymph reveals the fact that it is in every case higher than that in the venous blood. In one dog the lactic acid content of the lymph was found to be 106.9 mg. per 100 c.c. This animal was acutely ill, and also had a very low plasma chloride. The lymph on which the analysis was done was obtained within five minutes after the death of the animal, which probably accounts, at least in part, for the high figure reported.

TABLE I

DOG	BLOOD		LYMPH		DIFFERENCE IN CHLORIDES
	CHLORIDES	LACTIC ACID	CHLORIDES	LACTIC ACID	
Normal	--	10.0	--	14.8	
Pneumonia, 1	467.3	17.0	617.7	106.9	150.4
Pneumonia, 2	499.4	7.5	585.0	30.6	85.6
Pneumonia, 3	585.0	13.1	694.9	24.5	109.9
Pneumonia, 4	604.3	16.8	579.0	20.9	-25.3
Pneumonia, 5	585.0	11.0	655.0	15.8	70.0
Pneumonia, 6	604.3	9.9	599.6	13.3	- 4.7

*Gaseous Interchange in Pneumonia.*—From Tables I and II, it can be seen that because of the chloride decrease in pneumonia, the  $\text{CO}_2$  content of the blood tends to fall to compensate for this loss in acid element, and this action is reinforced by the lactic acid, which increases in amount. However, the  $\text{CO}_2$  content of the blood is often increased in pneumonia (Table III), because of improper

TABLE II

DOG	PNEUMONIA	TEMPERATURE	BLOOD				LYMPH			
			O <sub>2</sub> TENSION VOL. %	O <sub>2</sub> CAPACITY VOL. %	% SATURATED	CO <sub>2</sub> TENSION VOL. %	O <sub>2</sub> TENSION VOL. %	O <sub>2</sub> CAPACITY VOL. %	% SATURATED	CO <sub>2</sub> TENSION VOL. %
1	Rt. L.	102.8 sick	13.66	19.7	68.0	--	0.32	0.81	39	--
2	R. L. L.	103.5	17.49	--	--	--	0.271			
3	(30 min. be- fore death)	very sick	1.63	--	--	43.2	0.117			61.0
4	Rt. L.	102.8 not sick	--	--	--	--	0.884			
5	Rt. L.	102.4	17.00	--	--	--	0.45			40.0
6	Rt. L.	102.4 not sick	19.2	20.8	92.1	30.2	0.28	0.621	47	31.8

ventilation due to rapid breathing. It is then that an increase in lactic acid is important. Table V shows the results of oxygen therapy. Note that 1.3 per cent of oxygen is in the lymph, or almost twice as much as normal. This possibility of increasing oxygen supply to the cells, especially when anoxemia occurs in pneumonia, makes apparent the usefulness of oxygen therapy.

TABLE III  
EFFECT OF RAPID SHALLOW BREATHING IN PNEUMONIA

	BLOOD	LYMPH
O <sub>2</sub> Tension.	13.5 volumes per cent	0.42 volumes per cent
O <sub>2</sub> Capacity	20.0 volumes per cent	0.99 volumes per cent
O <sub>2</sub> Saturation	68.0 per cent saturated	41.00 volumes per cent saturated
CO <sub>2</sub> Tension	42.4 volumes per cent	64.00 volumes per cent

TABLE IV  
CHLORIDES, MG. PER 100 C.C.

	BLOOD SERUM	LYMPH (WHOLE)	EXCESS IN LYMPH
Dog 7	644	655	11
Dog 10	650	637	-13
Dog 11	663	737	74
Dog 14	640	682	42
Dog 15	649	667	18

TABLE V

NORMAL	BLOOD	LYMPH
O <sub>2</sub> Tension	19.5 volumes per cent	0.55 volumes per cent
O <sub>2</sub> Capacity	22.15 volumes per cent	0.73 volumes per cent
O <sub>2</sub> Saturation	88.00 volumes per cent (dog under ether)	75.00 volumes per cent
CO <sub>2</sub> Tension	42.40 volumes per cent After oxygen therapy	43.14 volumes per cent
O <sub>2</sub> Tension	-----	1.31 volumes per cent (more was forced in solution than it would normally hold)
CO <sub>2</sub> Tension	After CO <sub>2</sub> therapy (Dog breathed faster)	35.89 volumes per cent

*Chlorides.*—The figures for chloride concentration are tabulated for a series of five normal dogs. (Table IV.) In four instances the concentration in the lymph exceeded that in the blood plasma, the actual difference being from 11 to 74 mg. per 100 c.c. Of the analysis on the six dogs with pneumonia (Table II), in four instances the lymph chloride exceeded the blood concentration, being from 70 to 150 mg. per 100 c.c. more than the plasma chloride, the difference between the lymph and the blood plasma being higher in pneumonia than in normal dogs, both lymph and plasma chloride concentration were lower on the whole than were found in normal animals.

It seems, therefore, that the chlorides which are lost from the blood stream are not stored in the body fluid about the cells, because in such a case, the lymph, which is a part of this fluid, would have more chloride than normal, and this does not occur. What is more probable is that the exudation of blood elements during the consolidation of the lung carries with it the chlorides, which are therefore

lost to the general circulation and the cells. After the crisis the chlorides increase because of the absorption of the pneumonic process.

### SUMMARY

1. The lactic acid increase in pneumonia is a chemical phenomenon by which the loss of the chloride radical is neutralized. The decrease in  $\text{CO}_2$  content is another method, but too often, because of shallow breathing and improper aeration, this  $\text{CO}_2$  is increased in pneumonia. It is then that the increase in lactic acid is most important.

2. Oxygen therapy more than doubles the oxygen tension in lymph.

3. Chloride decrease in pneumonia is due to chloride retention in the exudation of the pneumonic lung. When consolidation disappears, chloride returns to the blood and lymph because of absorption of the pneumonic process.

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## EXPERIENCES WITH THE GRUSKIN SKIN TEST FOR THE DIAGNOSIS OF CANCER\*

JOSEPH MCFARLAND, M.D., JEFFERSON H. CLARK, M.D., AND MURRAY FRIEDMAN,  
M.D., PHILADELPHIA, PA.

AT THIS hospital a group composed of specialists in the various fields in medicine (internists, surgeons, gynecologists, pathologists, radiologists, etc.) cares for all cases of known or suspected malignancy, and meets regularly once a week to discuss the interesting or puzzling cases. It was the opinion of this group that the Gruskin skin test for the diagnosis of cancer should be given a trial to determine whether it is sufficiently reliable to be adopted as a routine diagnostic procedure. A committee of three, the authors of this paper, was, therefore, appointed to investigate its merits.

In order to escape a false start, Dr. Gruskin's cooperation was secured, and he kindly consented to furnish the necessary antigen. Throughout the investigation the tests were performed with 17 antigens made by him. Dr. Gruskin also gave several demonstrations of the correct technique of the test, familiarizing us with the fine pseudopods supposed to be characteristic of the positive reaction, and acquainting us with those conditions under which he believed the test to be unreliable. He was also good enough to interpret some of the early results.

*Technic.*—The technique resembles that employed in allergic work, although the resulting reactions are not at all comparable in intensity. A +++ Gruskin, for example, would be no more than a + reaction to ragweed pollen. The technique demonstrated by Gruskin was carefully followed; 0.2 c.c. of the first antigen, and 0.1 c.c. of the later antigens, said to be stronger and better because of the lessened bulk, were injected intradermally into the skin of the arm of the suspect with a 27 gauge hypodermic needle. From fifteen to twenty seconds were required for the injection and, in those cases regarded as positive, the blanched wheal resulting from the injection soon became reddened and very delicate pseudopods could be observed at its periphery in from five to ten minutes. The results were expressed as 0 or +, ++, +++, and ++++ according to the intensity of the reaction. A total of 199 tests upon 174 individuals was made, each test being checked by two observers.

*Control Cases.*—Control tests were made upon seventy-one patients (85 tests) suffering from a variety of chronic, nonmalignant diseases (see Table I) and 14 doctors and nurses in supposedly good health; 74.1 per cent of these tests proved negative. Of 22 positive reactions (including seven ++ and one +++) among the controls, 8 were in presumably healthy nurses. Two

\*From the Philadelphia General Hospital.  
Received for publication, June 15, 1934.

patients with parotid tumors gave negative reactions, while a third case of parotid tumor gave a negative reaction upon the first test and a ++ upon a later one. Negative tests were also obtained in one case of giant cell tumor, one of Hodgkin's disease and two of lymphatic leucemia.

TABLE I  
CONTROL SERIES

DIAGNOSES	CONFIRMATION	NUMBER CASES	NUMBER GRUSKIN TESTS	
			Neg.	Pos.
Normals	Clinical	13	11	8
Tuberculosis	X-ray	4	4	
Lupus vulgaris	Biopsy	1	1	
Syphilis	Wassermann	1		2
Aneurysm	X-ray	1	1	
Abscess	X-ray Oper.	3	2	1
Cellulitis	Clinical	1	1	
Arthritis	X-ray	1	1	
Endometritis	Biopsy	2	1	1
Cholecystitis	X-ray Oper.	2	2	
Osteomyelitis	X-ray	1	1	
Osgood-Schlatter's disease	X-ray	1	1	
Duodenal ulcer	X-ray	2	1	1
Chronic tonsillitis	Clinical	6	11	
Rectal stricture	Clinical	3	2	1
Abortion	Clinical	1		2
Chorea	Clinical	1		1
Hyperthyroidism	Clinical	1	1	
Intestinal adhesions	Oper.	2	2	
Fracture	X-ray	1	1	
Lymphatic leucemia	Blood	2	2	
Pernicious anemia	Blood	1	1	
Diabetes	Clinical	5	5	
Coronary disease	Clinical	3	4	
Cardiorenal disease	Clinical	2	2	
Macroductyly	X-ray	1		1
Warts	Clinical	1		1
Uterine fibroids	Clinical	1	1	
Osteoma	X-ray	1		1
Hodgkin's disease	Biopsy	1	1	
Giant cell tumor	X-ray	1	1	
Parotid tumor	Clinical	2	2	
Fibroadenoma of the breast	Biopsy	2		2
Totals		71	63	22
				74.1% Negatives

*Malignant Cases.*—These consisted of 103 known cases of cancer in which the diagnosis had been confirmed by a biopsy, or in which it was clinically evident through the occurrence of metastases, by observation at laparotomy or through x-ray examinations. Subgroups have been formed to contain cases of cancer confirmed by biopsies, which had had no radiation treatment and others also confirmed by biopsies, but which had received some form of irradiation.

We have conscientiously tried to follow Gruskin's directions in making the tests, avoiding all cases showing contraindications which result from altered metabolic states such as dehydration, jaundice, irradiation, and fever. Although Gruskin warns against performing the test in individuals shortly after treatment by x-ray or radium, we obtained a higher percentage of positive results in them than in those who had had no radiation therapy.

In 32 cancer cases confirmed by biopsy and already under x-ray or radium treatment, 34 tests gave 82.3 per cent positive and 17.7 per cent negative reactions. One case yielded an ambiguous result, and another differed in the strength of the reaction, according to the antigen used. In a group of 27 known cancer cases that had never received any form of irradiation, 32 tests gave 68.7 per cent positive and 31.3 per cent negative reactions. In a group of 20 cases, in which the diagnosis of cancer was clinical only, and in which there had been no irradiation, of 22 tests, 81.8 per cent were positive and 18.2 per cent negative. In 24 patients who had been irradiated and in whom the diagnosis of cancer was clinically outspoken, 26 tests gave 69.2 per cent positive and 30.8 per cent negative results (Table II). A total of 114 tests in 103 cancer cases gave 86 positive and 28 negative reactions. In the 86 positive tests there were only 21 ++ and three +++ reactions.

TABLE II  
MALIGNANT SERIES

	CASES	GRUSKIN POS.	TESTS NEG.	TOTAL TESTS	PER CENT POSITIVES
Carcinoma Confirmed by biopsy Irradiated	32	28	6	34	82.3
Carcinoma Confirmed by biopsy Not irradiated	27	22	10	32	68.7
Carcinoma Clinically confirmed Irradiated	24	18	8	26	69.2
Carcinoma Clinically confirmed Not irradiated	20	18	4	22	81.8
Totals	103	86	28	114	75.4

The average percentage of accuracy for the entire series of 199 tests, including both positive and control series, was 74.8 per cent (Table III). However, duplicate determinations were made in 10 individuals and in 3 of them the result changed from positive to negative or vice versa.

TABLE III  
TOTAL SERIES

	CASES	TOTAL TESTS		CORRECT RESULTS	PER CENT ACCURACY
Controls	71	85	Negatives	63	74.1
Carcinomas	103	114	Positives	86	75.4
Totals	174	199		149	74.8

*Intensifying the Reaction.*—As pointed out by Gruskin, difficulty is encountered in the application of the test in cachectic and dehydrated individuals. Many times a reaction suggesting malignancy appeared but disappeared so rapidly that it had to be read “plus-minus” and regarded as negative in the calculation for percentage accuracy. It was suggested by Dr. B. P. Widmann,

Chief Radiologist, that if wet dressings were continuously applied to the skin for twenty-four hours before the test, the local dehydration might be overcome, and the measure did seem to be of some value in the few cases in which it was tried.

The occurrence of an unusually marked reaction to the test in a patient who had just received an intravenous injection of "Synadol," a proprietary remedy containing emetine and various lipoids, suggested that it might be used as a means of intensifying the reaction. The Gruskin antigen itself was tried as an intensifying agent by immediately following the endodermal injection of the antigen by a subcutaneous injection of 0.5 c.c. of the same antigen. Within twenty-four hours the patient experienced itching of the skin and repetition of the intradermal test performed at that time produced more distinct reactions in several cases. In one case a typical urticaria, localized to one arm, followed the second test. Control cases did not react similarly. We are unable to explain satisfactorily the intensification by these means, for the interval between the injection and subsequent test is too short to permit true tissue sensitivity to develop.

*Discussion.*—Unquestionably any test which will aid in the early diagnosis of cancer should be a distinct adjunct in the institution of early treatment, and so assist in the cure of the disease. Such a test, however, should be so simple as to be easily performed and so reliable as to be superior to all other diagnostic measures. From our experience the "Gruskin skin test for cancer" does not fulfill these specifications.

Gruskin declares this to be an "allergic reaction." He supposes that tumors consist of embryonal cells that are foreign to the body of the host, presumably because all embryonal cells should eventually cease vegetating, mature, differentiate and become useful and specialized members of the body. In neoplasia there is supposed to be an anaplastic return to the embryonal state as shown by the unlimited vegetative cellular activities, and the multiplying cells have been shown to have unusual metabolic requirements and to give off unusual metabolic products as compared with adult specialized cells. These products, resembling those of growing embryonal cells, are also supposed to be abnormal to the adult body, and entering the blood to effect a kind of sensitization and the formation of an antibody that is supposed to be a factor in the reaction constituting the test.

He also assumes that various types of embryonal cells emit such differing chemical sensitizing products ("foreign protein of embryonic character") as to permit of the identification of different types of tumor by the use of antigens made from different types of embryonal tissue. For example, for the identification of cancer the so-called antigen is made from embryonal liver, for sarcoma from umbilical cord.

We cannot agree that there are essential differences between embryonal and adult cells other than can be accounted for by their differing activities, growth and multiplication preponderating in the former, and secretion, contraction, nerve impulse transmission, etc., in the latter. For these activities slightly different quantities and qualities of foods may be required, and from them

slightly different qualities and quantities of waste products may be given off, but all of them are native to the body by which they are furnished and easily dealt with by the body accustomed to get rid of them. It is, therefore, not to be imagined that their presence in an infinitesimal amount could effect a "sensitization" of the whole body to the future admission of an antigen supposed to be essentially the same material.

It is also entirely in error to suppose that cell multiplication is indicative of embryonal character in the sense used by Gruskin. There are always multiplying cells in the epiderm, and considering the ten or more square feet of the body covering, their number should be quite as great as that found in a skin cancer, yet they effect no sensitization. Each month the formation of new parenchyma in the female breast necessitates an enormous cell proliferation resulting in as many cells as would be found in a small mammary cancer, yet without any sensitization. As Gruskin represents the test as useful for the diagnosis of cancer so early in its development as to escape detection by other methods, it may be inferred that an extremely small number of cells may be sufficient to effect the sensitization. How many cells are required to do that? If an extremely small number will suffice, why do negative results frequently occur when large cancers are present?

Noting that patients in the best of health occasionally give positive reactions, and that one or two of his cases, behaving in this paradoxical fashion, were the descendants of cancerous ancestors, Gruskin concludes that the test not only reveals the presence of existing cancer, but also may foretell the future probability of cancer. In other words, the test that is to be depended upon for the immediate diagnosis of the disease may only be indicating its future and remote possibility. Such an unfortunate admission destroys the value of the test. We want to know whether the patient has cancer now, not that he may get it twenty years hence. As out of any number of normal persons questioned, some would undoubtedly be found to descend from carcinomatous ancestors, the assumption that such descent is sufficient to explain the paradoxical positive reactions in the healthy, seems to be no more than a forced attempt to escape from a difficulty.

But this is not all; the reactions of immunity and allergy are among the most specific known, so it becomes necessary to consider carefully the so-called antigens employed in the prosecution of the test. According to the directions given by Gruskin, they are prepared from the embryonic livers of calves, sheep or pigs in the second month of fetal life. The liver substance is separated from its capsule, washed in water to remove as much blood as possible, and then vigorously shaken with water in an Erlenmeyer flask to separate the cells from the fibrous tissue. The suspension of cells is then centrifuged at high speed and the cells repeatedly washed with water until free from all traces of blood. The wet cells are then rubbed up with twenty volumes of N/10 NaOH, allowed to stand for twenty-four hours, after which the reaction is adjusted to pH 6.8 with N/10 HCL.

From the experiences of our friends and ourselves, this "antigen" is extremely difficult to make and its originator finds it no less so. In the first place one should not be deceived into supposing that it can be satisfactorily made of



the livers of pigs. When so made it usually will not work, probably because of the presence of "too much fat." To make it from other livers is difficult because little bovine and ovine embryos are very difficult to get. But when all of these difficulties are overcome, there is no method of titration by which its usefulness can be measured or the quantity necessary to produce the reaction determined. Gruskin himself frequently said that the antigens were "no good" or "too weak," and from him came a succession of 17 antigens, some of which when used on patients previously tested, gave results contrary to those obtained with another antigen.

The antigen is, therefore, too variable a reagent to be depended upon, but it is not only variable, it is also fantastic. We have told what it is, but let us now see how it is supposed to act. On very doubtful grounds, it is assumed that cancer cells through anaplasia become embryonal cells. Although originally homologous, they now become rebellious aliens, resented and combated by the normal structures of the body by which they are surrounded. This, it is further assumed, results in such a disturbance of the whole body that it not only becomes keenly sensitive to their presence and products, but it also becomes sensitive to exogenous heterologous proteins derived from animals of such various and widely divergent types as cattle, sheep, and hogs.

This concept seems to be so contrary to all of the known facts of blood relationship, specific precipitation and allergy as to be unimaginable. If the extracted protein, constituting the "antigen" is an embryonal extract, if cancer cells are also embryonal, and if the latter can be conceived to have sensitized the body to embryonal substance, it would not follow that the former would react with the latter. The embryonal extract used in the "antigen" is more than an embryonal extract; it is an embryo *sheep* extract or *calf* extract or *pig* extract. The phylogenetic divergence of these animals is from the very beginning. The ovum of an animal of one group cannot be fertilized by the spermatozoa of another group; the tissues of one kind, whether embryonal or adult, will not live if transplanted into another kind; the blood of one kind cannot by transfusion support the life of another kind.

But Gruskin's imagination goes still further; the antigen for cancer diagnosis is an epithelio-antigen and its alleged specific reaction is demonstrable only in the case of malignant tumors, because their cells acquire and maintain an embryonal character through anaplasia. It will not react in the case of benign epithelial tumors, because these cells not being anaplastic and embryonal, differentiate and mature. If there is any tumor that contains embryonal epithelium, it is the mixed tumor of the parotid, yet it does not consistently react positively.

Should it be desired to make a diagnosis of sarcoma, it becomes necessary to employ a connective tissue antigen. To meet this requirement Gruskin has selected the umbilical cord as the appropriate embryonal source of the antigen, preparing it in much the same way as was described above.

But is the umbilical cord an embryonal tissue in the sense in which Gruskin uses that term? It is a completed and perfect structure, does not continue to grow beyond a certain point and never produces anything other than itself. At

the time of birth it is already mature, inactive and its cells decadent. Does it, therefore, conform to the requirements for the antigen? But if it be embryonal in the required sense, is it of a quality specific for reacting with embryonal connective tissue only? The umbilical cord is composed of an ectodermal membrane, amnion, on the outside; in it are vestiges of the yolk sac and allantois, both endodermal and all that remains are the blood vessels and jelly of Wharton, the former mesodermal, the latter uncertain in origin. With a mixture of ectodermal, mesodermal and endodermal derivatives, how can it be specific for supposedly embryonal mesoblastic tumors, sarcomas?

But behind all this lies a more profound biologic defect, for the whole scheme is founded upon certain assumptions that are oncologically unsound. We have become accustomed to employ the words "carcinoma" and "sarcoma" so familiarly that they have come to imply more knowledge than is justified by the facts. Carcinomas and sarcomas are clearly separated in theory but not in fact. The sarcoma group is extremely vague and puzzling and has been subject to many subtractions in recent years, and will be subject to many more, no doubt. For example, melanotic sarcoma has ceased to exist because of the incompatibility of the usage of a name (sarcoma) usually implying a tumor of mesodermal origin, for a tumor that is almost certainly of ectodermal origin; of the tumors of the lungs and prostate formerly called sarcomas, many are now regarded as carcinomas. Almost the whole oncological classification and nomenclature is in a confused state that affords such evidence of present ignorance of the origin and nature of the tumors themselves as to make it impossible that such specificity as the Gruskin test presupposes can obtain.

#### PRACTICAL QUESTIONS

The adequacy and inadequacy of the test may be judged by the way in which it seems advisable to answer the following questions:

1. In how many cases can the diagnosis of cancer be correctly made by the Gruskin test when all other methods of diagnosis have failed? The number must be very small for of our 27 cases proved by biopsy to be suffering from cancer, not yet subjected to irradiation by x-rays or radium and appropriate for the test from every point of view, only 68.7 per cent reacted positively.

2. In case of a suspected invisible internal cancer should the patient be advised to undergo a laparotomy or other operation because the Gruskin test is positive? In view of the fact that out of 14 young persons in perfect health and above any suspicion of cancer, 8 gave positive reactions, the answer to this question ought to be an emphatic NO.

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## OBSERVATIONS ON INTESTINES OF RATS FED INERT MATERIALS\*

CHARLES-FRANCIS LONG, M.D., JOHN A. KOLMER, M.D., AND WILLIAM A. SWALM,  
M.D., PHILADELPHIA, PA.

FOR centuries inert materials notably kaolin, have been added to the diet of human beings suffering from diarrhea of various sorts.<sup>1</sup> Long empiric history and continuing use would bespeak the fact that these inert bodies fulfill their purposes in the hands of physicians. In the recent medical literature untoward effects have been reported through using kaolin in the human being and in the experimental animal.<sup>2</sup>

This paper will report laboratory studies conducted with white rats, observing the effects of various inert materials upon their intestinal tracts after varying lengths of time. Rats were chosen because they will eat almost anything, and diets analogous to the human being could be constructed for them. Furthermore the most severe criticisms of large doses of kaolin as a medicament have come from the rat feeding experiments of Fredrick Hoelzel of Chicago.<sup>3</sup> He points out that in many instances the admixture of kaolin in the proportion of 2 to 1 with the diet causes polyposis of the intestinal mucosa. He believes the mechanism of their production to be as follows:

The kaolin causes retarded movement of the fecal mass along the gut, resulting in long contact with one section of the mucous membrane. Gas pockets form in the feces which move toward the periphery and cause a dimpling or pitting of the fecal surfaces. As this gas is absorbed or slips out the mucous membrane is sucked into or falls into the pit and thus forms a polyp providing there is a lapse of from six to twenty-four hours in which the fecal mass remains in situ. According to Hoelzel one-third of the rats on such a regime may develop sessile or pedunculated polyps.

Through the kindness of Dr. John A. Kolmer and the Research Institute of Cutaneous Medicine, rats were made available for a similar study in a small series of animals, using not only plain kaolin but in addition kaolin in a suspension of aluminum hydroxide gel, cellulose and bran. One obvious criticism of the work of Hoelzel is the overwhelming amount of inert material used in some of his experiments, so we also observed the effects of dosage more nearly commensurate with human dosage on the basis of average weights. At the end of the experiment using 87 rats, we found several objections to the results which will be pointed out later, so a second group of 27 rats was observed for four weeks to check the moot points in the first group.

We accepted for the purpose of the experiment Hoelzel's conception that pitting of the rat feces indicates gas pocketing during stasis and potential

\*From the Gastro-Intestinal Clinic of the Department of Medicine, Temple University Medical School and the Research Institute of Cutaneous Medicine, Dr. John A. Kolmer, Director.  
Received for publication, June 15, 1934.

mucosal harm, but we have relied solely on autopsy findings for conclusions as to the harmfulness or harmlessness of the inert bodies. Our work lends itself rather well to tabulation which will be more informative than a long written account. The diets used in the first group of rats were as follows:

- |  |  |
|--|--|
| <p>(1) Hoelzel Diet:</p> <p>30 Parts protein (com'l casein)</p> <p>3 Parts cod liver oil (fat and vitamin A)</p> <p>5 Parts dry yeast powder (vitamin B)</p> <p>60 Parts cornstarch</p> <p>2 Parts NaCl</p>  | <p>(3) McCollum's Stock Diet:      Semi-Rough</p> <p>Whole wheat flour                      65.5%</p> <p>Casein                                      15.0%</p> <p>Whole milk food                        12.0%</p> <p>Butterfat                                  5.2%</p> <p>Calcium carbonate                    1.5%</p> <p>NaCl                                        0.8%</p> |
| <p>(2) Smooth Diet (Weirda's):</p> <p>18 Parts protein (com'l casein)</p> <p>23 Parts lard or butterfat (lard used)</p> <p>53 Parts sugar or cornstarch (cornstarch used)</p> <p>4 Parts Osborne and Mendel's salt mixture</p> <p>Supplement with 1 gm. yeast daily</p> <p>Supplement with 5 drops cod liver oil daily</p> | <p>(4) Normal Rat Diet (Research Institute):</p> <p>Buckwheat                                25%</p> <p>Oats                                        40%</p> <p>Rolled oats                               21%</p> <p>Sunflower seed                        4%</p> <p>Cracked corn                            10%</p>  |

## OBSERVATIONS

1. *Rate of Fecal Passage.*—The addition of massive doses of inert materials to the diet of rats, as near as possible equal in weight, resulted in a *definite increase in the number of fecal pellets passed as compared with the control series* (compare with Table VI). In this respect the materials used stand as follows: (1) powdered cellulose (Table III); (2) kaolin (Table I); (3) flaked cellulose (Table III); (4) bran (Table V); (5) kaolin in aluminum hydroxide gel (Table

TABLE I

NO.	DIET DAYS	FECES	PITTING	PARASITE	WEIGHT GAIN + LOSS -	AUTOPSY FINDINGS
<i>A. 1 Part Hoelzel Diet Plus 2 Parts Kaolin</i>						
1	33	717	143 (19.9%)	In liver	-30 gm.	No lesion
2	38	833	147 (17.5%)	In small intestine	- 8 gm.	No lesion
3	46	1,081	180 (16.6%)	In small intestine	+25 gm.	No lesion
4	59	1,369	214 (15.6%)	0	-14 gm.	No lesion
5	73	1,607	262 (16.3%)	In small intestine	+ 5 gm.	No lesion
6	24	485	100 (20.6%)	0	- 3 gm.	Hemorrhagic ulcer large intest.
Summary:		6,092	1,046 (17.2%)	4		
<i>B. Hoelzel Diet Plus 0.9 Gm. Kaolin (Human Rat Dose)</i>						
1	34	114	3 (2.6%)	0	+31 gm.	No lesions
2	38	161	0	Small intestine	+42 gm.	No lesions
3	46	267	2	0	+32 gm.	No lesions
Summary:		542	5 (0.9%)	1		
<i>C. Hoelzel Diet Plus 1.8 Gm. Kaolin (Human Rat Dose)</i>						
4	23	80	2 (2.5%)	0	+42 gm.	Died, no lesions
5	52	190	4 (2.1%)	0	+12 gm.	No lesions
6	73	359	3 (0.8%)	In liver	+58 gm.	No lesions
Summary:		629	9 (1.4%)	1		

IV). However, it will be easily noted that when the dosages were reduced to those used in human clinical practice, the rate of fecal passage in every instance was normal. The faster fecal rate is possibly a function of both increased water adsorption and mucosal stimulation.

TABLE II

NO.	DIET DAYS	FECES	PITTING	PARASITE	WEIGHT GAIN + LOSS -	AUTOPSY FINDINGS
<i>A. 1 Part Normal Diet Plus 2 Parts Kaolin (Four Days Only) Then 1½ Parts Normal Diet Plus 1½ Parts Kaolin, and Carrot Twice Weekly</i>						
1 (a)	5	545	56 (10.2%)	0	- 5 gm.	<i>Died of heat and inanition. No lesion</i>
1 (b)	20					<i>No lesions</i>
2	38	766	93 (10.8%)	0	+15 gm.	<i>No lesions</i>
3 (a)	4	1,504	198 (13.1%)	0	0	<i>Died of heat and inanition. No lesion</i>
3 (b)	21					<i>No lesion</i>
Summary:		2,815	347 (12.4%)	0		
<i>B. 1 Part Normal Diet Plus 1 Part Kaolin Plus a Carrot Twice Weekly</i>						
4	46	875	99 (10.2%)	0	+22 gm.	<i>No lesion</i>
5 (a)	5	931	111 (11.8%)	Small intestine	-42 gm.	<i>Died of heat and inanition</i>
5 (b)	38					<i>No lesion</i>
6 (a)	5	1,094	104 ( 9.0%)	Small intestine	-54 gm.	<i>Died of heat and inanition</i>
6 (b)	21					<i>Died. No lesion</i>
Summary:		2,900	314 (10.8%)	2		

SUMMARY OF TABLE III

- A. Six rats fed 1 part Hoelzel diet plus 2 parts *powdered* cellulose for a combined period of 116 days passed 3,951 feces of which 60 (1.5 per cent) were pitted. One polyp was found situated in the large intestines. Two parasites were found.
- B. Twelve rats fed 1 part Hoelzel diet plus 2 parts *flaked* cellulose for a combined period of sixty-two days passed 940 feces without pitting. No lesions were found but there were 5 parasites.
- C. Three rats fed 9 parts Hoelzel diet plus 1 part flaked cellulose for a combined period of forty-five days passed 419 feces without pitting or lesions. Two parasites were found.
- D. Three rats fed 8 parts Hoelzel diet plus 2 parts flaked cellulose for a combined period of fifty-two days passed 462 feces without pitting or lesions. One parasite was found.

2. *Production of Pitted Feces.*—There was no pitting of the feces of the control rats on the Hoelzel and normal diets (Table VI), laying at rest our anxiety that the pitting might be really indentations made by the rat's feet. Nor was pitting seen in those rats receiving flaked cellulose (Table III) or bran (Table V). Curiously enough one rat each on Weirda's diet and McCollum's diet showed a slight amount of pitting (Table VI).

Pitting was most marked after massive doses of kaolin (17 per cent, 12.4 per cent, 11 per cent) (Tables I and II). Second to plain kaolin stood kaolin

in aluminum hydroxide gel (7.4 per cent) (Table IV). Powdered cellulose caused only 1.5 per cent pitting (Table III).

When the dosage of inert material was reduced to more nearly the proportionate human dose, pitting was reduced to 1 per cent and 1.4 per cent for kaolin (Table I) and 0.5 per cent and 0.0 per cent for kaolin in aluminum hydroxide gel (Table IV).

3. *Production of Lesions.*—Our results were in marked contrast with those of Hoelzel. No polyps were produced in any of the kaolin studies. The single polyp discovered occurred in a rat on one part Hoelzel diet plus powdered cellulose 2 parts, autopsied on the thirty-ninth day (Table III). Three hemorrhagic ulcers of the large intestine were discovered (Tables I, IV, and VI), *but since one occurred in a control rat*, the inert material in the diet must be exonerated in their production.

At the conclusion of the experiment it was found that 35 of the 87 rats were infested with a nematode parasite and that the arithmetical calculations of the smaller dosages had been inaccurate. Consequently, it was thought wise to re-study the problem of kaolin and kaolin in aluminum hydroxide gel using a series of 27 carefully selected rats, including new controls. The rats were provided by the Wistar Institute of Anatomy in Philadelphia. They were carefully

TABLE IV

NO.	DIET DAYS	FECES	PITTING	PARASITES	WEIGHT GAIN + LOSS -	AUTOPSY FINDINGS
<i>A. 1 Part Hoelzel Diet Plus 2 Parts Kaolin 20 Per Cent in Aluminum Hydroxide Gel</i>						
1	34	375	34 (8.6%)	0	+17 gm.	Ulcer in large intestine
2	38	334	25 (7.7%)	0	+24 gm.	No lesions
3	46	460	39 (8.4%)	0	+57 gm.	No lesions
4	52	529	39 (7.3%)	0	+33 gm.	No lesions
5	59	532	34 (6.5%)	0	+24 gm.	No lesions
6	73	688	46 (6.6%)	0	+76 gm.	No lesions
Summary:		2,918	217 (7.4%)	0		
<i>B. 1 Part Hoelzel Diet Plus 1.8 c.c. Kaolin 20 Per Cent in Aluminum Hydroxide Gel (Double Human Rat Dose)</i>						
1	34	147	0	0	+12 gm.	No lesions
2	38	159	1 (0.07%)	In small intestine	+42 gm.	No lesions
3	62	252	2 (0.3%)	0	+68 gm.	No lesions
Summary:		558	3 (0.5%)	1		
<i>C. 1 Part Hoelzel Diet Plus 3.6 c.c. Kaolin 20 Per Cent in Aluminum Hydroxide Gel (Quadruple Human Rat Dose)</i>						
4	38	120	0	0	+26 gm.	No lesions
5	52	178	0	0	+57 gm.	No lesions
6	52	363	0	In liver	+78 gm.	No lesions
Summary:		661	0	1		

SUMMARY OF TABLE V

- A. Three rats fed one part Hoelzel diet plus 2 parts prepared bran for a total of sixty-one days passed 725 feces without pitting or lesions. One parasite was found.
- B. Three rats fed 1 part Hoelzel diet plus 2 parts milled bran for a total of thirty-two days passed 273 feces without pitting or lesions. All had parasites.

TABLE VI  
FIRST SERIES

NO.	DIET DAYS	PECES	PITTING	PARASITES	WEIGHT GAIN + LOSS -	AUTOPSY FINDINGS
<i>A. Control, Smooth Diet (Weirda)</i>						
1	7	78	0	In liver	+ 6 gm.	No lesions
2	14	49	0	0	+ 5 gm.	No lesions
3	24	92	1 (1.1%)	In liver	?	No lesions
Summary:		219	1 (0.5%)	2		
<i>B. Control, McCollum's Stock Diet</i>						
4	7	67	0	In liver	+ 6 gm.	No lesions
5	14	68	0	0	+23 gm.	No lesions
6	24	327	2	In liver	+25 gm.	No lesions
Summary:		462	2 (0.4%)	2		
<i>C. Control, Smooth Diet (Weirda) Plus Iron Citrate</i>						
1	4	27	0	0	-	No lesions
2	12	71	0	0	+ 4 gm.	Ulcer of cecum
3	12	80	0	0	+ 7 gm.	No lesions
Summary:		178	0	0		
<i>D. Control, Milk Twice Daily Plus 3 Drops Viosterol (Six Days), Then 1 Drop Viosterol (Six Days)</i>						
4	12	32	0	In small intestine	-17 gm.	No lesions
5	4	18	0	Intestine	-	No lesions
6	12	32	0	0	-16 gm.	No lesions
Summary:		82	0	2		
<i>E. Control, Hoelzel Diet</i>						
1	15	56	0	Small intestine	+ 4 gm.	No lesions
2	23	90	0	0	-11 gm.	No lesions
3	29	101	0	Small intestine	+37 gm.	No lesions
4	36	117	0	In liver	+27 gm.	No lesions
5	42	120	0	0	+11 gm.	No lesions
6	49	202	0	0	+30 gm.	No lesions
Summary:		686	0	3		
SECOND SERIES						
<i>A. Control, Normal Laboratory Rat Diet</i>						
1	3	17	0	0	+ 4 gm.	No lesions
2	10	92	0	0	- 3 gm.	No lesions
3	16	123	0	0	- 8 gm.	No lesions
4	23	150	0	0	-23 gm.	No lesions
Summary:		382	0	0		
<i>B. Controls, Hoelzel Diet</i>						
1	28	169	0	0	+ 9 gm.	Negative
2	28	136	0	0	- 1 gm.	Negative
3	28	132	0	0	-31 gm.	Gas distention small intestine
4	28	126	0	0	-49 gm.	Gas distention small intestine
5	28	132	0	0	No gain or loss	Negative
6	28	208	0	0	- 9 gm.	Negative
Summary:		903	0			
<i>C. Wistar Diet Controls, Wistar Diet</i>						
1	28	557	0	0	+55 gm.	Negative
2	28	356	0	0	+52 gm.	Negative
3	28	340	0	0	+53 gm.	Negative
Summary:		1,253	0	0		

TABLE VII

NO.	DIET DAYS	FECES	PITTING	PARASITES	WEIGHT GAIN + LOSS -	AUTOPSY FINDINGS
<i>1 Part Hoelzel Diet Plus 2 Parts Kaolin</i>						
1	28	1,141	74 (6.4%)	0	-18 gm.	Negative
2	28	944	94 (1.01%)	0	-26 gm.	Cyst in liver
3	28	970	59 (6.0%)	0	-22 gm.	Negative
Summary:		3,055	227 (7.0%)	0		
<i>Hoelzel Diet Plus 0.140 Gm. Kaolin Per Rat Per Day (Double Human Rat Dose)</i>						
1	28	198	0	0	+ 1 gm.	Negative
2	28	184	3 (1.6%)	0	+ 3 gm.	Negative
3	28	150	0	0	- 5 gm.	Negative
Summary:		532	3 (0.5%)	0		
<i>Wistar Diet Plus 0.140 Gm. Kaolin Per Rat Per Day (Double Human Rat Dose)</i>						
1	28	473	0	0	+45 gm.	Negative
2	28	406	0	0	+42 gm.	Negative
3	28	409	0	0	+37 gm.	Negative
Summary:		1,288	0	0		

TABLE VIII

NO.	DIET DAYS	FECES	PITTING	PARASITES	WEIGHT GAIN + LOSS -	AUTOPSY FINDINGS
<i>1 Part Hoelzel Diet Plus 2 Parts Kaolin 20 Per Cent in Aluminum Hydroxide Gel</i>						
4	28	568	28 (4.0%)	0	+13 gm.	Polyp
5	28	608	31 (5.0%)	0	- 1 gm.	Negative
6	28	632	21 (3.0%)	0	-13 gm.	Negative
Summary:		1,808	80 (4.0%)	0		
<i>Hoelzel Diet Plus 9 Drops Kaolin 20 Per Cent in Aluminum Hydroxide Gel Per Rat Per Day (Double Human Rat Dose)</i>						
4	28	206	1 (0.4%)	0	+ 1 gm.	Negative
5	28	141	0	0	- 8 gm.	Cyst in liver
6	28	220	1 (0.4%)	0	-16 gm.	Negative
Summary:		567	2 (0.3%)	0		
<i>Wistar Diet Plus 9 Drops Kaolin 20 Per Cent in Aluminum Hydroxide Gel Per Rat Per Day (Double Human Rat Dose)</i>						
4	28	365	0	0	+28 gm.	Negative
5	28	437	0	0	+46 gm.	Cyst in liver
6	28	344	0	0	+39 gm.	Negative
Summary:		1,146	0	0		

checked for ova and parasites in the feces before commencing with the experiment and in addition to the Hoelzel diet, a control series was placed on the Wistar diet as follows:

Wistar Institute rat food Mixtures 1, 2, and 3 were used in succession. These rat food mixtures consist of fish, meat, and fresh vegetables. In addition milk, cod liver oil, lettuce, and hard boiled egg were fed three times per week. All rats in this series lived to and were autopsied on the twenty-ninth day.

## OBSERVATIONS

1. *Rate of Fecal Passage.*—The same speeding up of fecal rate was noted in this as in the previous series when massive doses of the inert bodies were used



# HEMOGRAPHY—CONTROLLED NONSPECIFIC IMMUNOTRANSFUSIONS IN THE TREATMENT OF SEPTICEMIA AND OTHER ACUTE INFECTIONS\*

WALTER J. CROCKER, V.M.D., E. H. VALENTINE, M.D., AND  
WILLIAM BRODY, M.D., PHILADELPHIA, PA.

WE ARE presenting a preliminary report on fifty-two cases of septicemia and acute infections treated with hemography-controlled nonspecific immunotransfusions, suggested by the work of Wright<sup>1</sup> and Colebrook and Sterer.<sup>2</sup>

The object of this paper is threefold:

1. To show that cases, in which this treatment is indicated, can be diagnosed and controlled best when shiftograms are used in conjunction with clinical findings and other laboratory data.

2. To show that nonspecific immunotransfusions may be used successfully in the treatment of acute infections which are of such rapid progress and great severity that there is not time to prepare a specifically immunized donor, vaccine or other biologic substance.

3. To point out the fact that unless there is adequate natural or surgically established drainage of the foci of infection, the beneficial effect of the non-specific immunotransfusions is only transitory.

All the cases treated with nonspecific immunotransfusions are from the various wards of the Philadelphia General Hospital except one from the surgical practice of J. A. Fitzmaurice. All cases are common acute infections of unusual severity, where clinical findings and laboratory data, such as blood cultures, and hemography have led to a doubtful prognosis.

Daily hemograms were done and charted and each case was closely controlled by hemograms before and after transfusion. In this manner a change of the patient's condition for better or worse was often detected several hours before it had appeared clinically. A second donor could then be prepared for transfusion while the patient's condition was still fair.

This method of treatment has been used with excellent results in such acute general infections as pneumococci and streptococci pneumonia; post-operative septicemia; meningitis; hemolytic streptococci septicemia; puerperal and postabortal septicemia. It should be remembered that a nonspecific immunotransfusion and the elimination of the primary focus of infection and adequate drainage is a factor of equal im-

(compare Tables VII and VIII). When the dosage was reduced to that proportionate to the human being, the rate was again normal.

2. *Pitting of Feces*.—Using massive doses of kaolin, the pitting was 7 per cent; using kaolin in aluminum hydroxide gel, the pitting was 4 per cent. No pitting was seen in rats fed either substance in amounts calculated to be double the human dose on *Wistar diet* (Table VII), but pitting was observed in 0.5 per cent and 0.3 per cent of feces in rats on this same dosage with the *Hoelzel diet* (Table VII).

3. *Production of Lesions*.—One polyp was found after twenty-eight days in a rat fed one part Hoelzel diet plus two parts kaolin in aluminum hydroxide gel (Table VIII). No parasites were found, but there were three liver cysts.

#### DISCUSSION

We have not been able to produce lesions as frequently as Hoelzel, since we found only two polyps in a total of 114 rats autopsied. We cannot agree that pitting occurs during fecal stasis, for it was most marked where the fecal rate was fastest. We do agree with Hoelzel that massive doses of any inert bodies may produce lesions in the intestines of rats, but we find no instance from our work in which this occurred in dosage double that used in ordinary human therapy.

#### SUMMARY

1. It is stated (Hoelzel) that feeding large amounts of kaolin and other inert substances predisposed to pathologic effects in the intestinal mucosa with special reference to the production of polyps.

2. Rats fed various inert bodies in large and small doses were autopsied at varying intervals together with suitable controls.

3. The results warrant the statement that massive doses of any inert bodies speed the rate of fecal passage; using small doses this rate remains normal.

4. Pitting of feces was marked when using massive doses of kaolin, kaolin in aluminum hydroxide gel and powdered cellulose; bran and flaked cellulose caused no such results.

5. But pathologic lesions were not produced with sufficient regularity to warrant the assumption that these substances produce intestinal polyposis. Certainly they do not produce these changes when given in amounts equivalent to the doses administered to human beings.

The authors wish to gratefully acknowledge the technical assistance of Miss Katherine Borden and Miss Edith Heller.

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256 SOUTH TWENTY-FIRST STREET  
TWENTY-FIRST AND PINE STREETS  
4901 NORTH THIRTEENTH STREET

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This method of treatment has been used with excellent results in such acute general infections as pneumococci and streptococci pneumonia; post-operative septicemia and peritonitis; hemolytic streptococci septicemia; puerperal and postabortive sepsis. It should be remembered that a nonspecific immunotransfusion is not a cure-all and the elimination of the primary focus of infection, or the establishment of adequate drainage is a factor of equal importance in every case in which it is used.

\*From the division of Clinical Pathology of the Laboratories of the Philadelphia General Hospital.

Received for publication, July 21, 1934.

It should be noted here that we have never seen any unfavorable or severe reactions due to the use of the nonspecific immunotransfusion, no matter how weak the patient's condition.

#### APPLICATION OF HEMOGRAPHY AND RESULTS OF THERAPY

Of the 52 cases in which hemography was used to control nonspecific immunotransfusion therapy, 25 recovered and 27 died (Chart 1). This does not represent the ratio of efficiency of this form of treatment, as autopsy findings in many of the fatal cases showed them to be incurable.

The same general clinical types of disease appear in each group. Septicemia, pyemia, or infection was diagnosed by hemogram in all cases before the blood was cultured. Of the first group blood culture was positive in 7 cases; *Streptococcus hemolyticus* in 5, *Staphylococcus albus* in 1, and pneumococcus Type I in 1 case. In the second group blood culture was positive in 13 individuals; *Streptococcus hemolyticus* in 4, *Staphylococcus albus* in 5, *Staphylococcus aureus* in 1, and pneumococcus Type IV in 3 cases.

The hemogramic prognosis was equally bad in all cases before nonspecific immunotransfusion was begun. Twenty-four to forty-eight hours after the last treatment the hemogramic prognosis was good in the 25 patients that lived, as the average right shift and number of multiples gained suggested convalescence. In the second group, or fatal cases, the hemogramic prognosis twenty-four to forty-eight hours after the last transfusion was generally bad. In 8 of these cases the shift was to the left with a loss of multiples in spite of the transfusions. In the remainder of the second group immunotransfusion was followed by a right shift with a reduction of the average multiple index per transfusion, but the initial left shift had been so wide that this improvement made little change in the prognosis, since the ground thus gained was rapidly lost. This may be explained by the presence of an overwhelming infection feeding toxins into the blood stream so rapidly that the opsonins could affect them only temporarily.

In estimating the value of nonspecific immunotransfusion therapy, it is only fair to point out that of the 27 patients that died:

1. Six were incurable; subhepatic abscess, acute reticuloendotheliosis, aplastic anemia, aleucemic myelosis, lymphoblastoma of the intestine and sub-acute bacterial endocarditis.

2. Fourteen cases were complicated by other serious conditions: ruptured appendix by diffuse peritonitis; cellulitis with *Staphylococcus albus* septicemia, by tuberculoma of the brain and diabetes mellitus; gangrene of the foot, with *Staphylococcus albus* septicemia, by diabetes mellitus; mastoiditis by lateral sinus thrombosis; bronchopneumonia by adenocarcinoma of the ileum; lobar pneumonia (two cases) by uremia; lobar pneumonia by cardiac decompensation; bronchopneumonia (two cases) by lung abscesses; bronchopneumonia by empyema; perforated duodenal ulcer by diffuse peritonitis; septic metritis (two cases) by diffuse peritonitis.

3. The remaining 7 cases were either uncomplicated or no autopsy was performed.

The hemogram is a horizontally arranged line of figures representing the various items of interest pertaining to the blood picture.

The "shiftograph" (see shiftograms) is a vertical graphic representation of the Schilling indices of sequential hemograms arbitrarily adopted by us to express, best, the extent of the quantitative shift and its relation to the qualitative shift, or the types of neutrophilic cells appearing in the peripheral blood.

The "shiftogram" is a chart composed of sequential hemograms connected by a "shiftograph," showing the entire course of the disease.

#### PREPARATION OF DONOR

A suitable donor is selected by typing, cross-agglutination and serology. He is put to bed and given fifty million\* killed typhoid organisms intravenously. The commercial antigen, either typhoid or typhoid and paratyphoid A and B used commonly for inoculation and foreign protein shock therapy, may be used. Within an hour following the injection the donor's symptoms set in, with headache, malaise, chills and fever, and occasionally nausea, vomiting and diarrhea. The temperature often reaches  $104^{\circ}$ , but rapidly falls to normal. If no reaction occurs within an hour an additional twenty-five million organisms should be given intravenously. The transfusion is performed at the height of the hematologic reaction which occurs in about eight hours. Though the donor's reaction may be of some severity, he returns to normal in twenty-four hours. During the reaction the donor's temperature rises to  $100-104^{\circ}$ ; his leucocyte count increases from normal to 15,000 to 25,000 and his hemogram shows a strong regenerative, degenerative left shift which returns to normal in two or three days. A donor should not be used a second time in less than two weeks. Blood drawn from a donor, in citrate, may be kept safely on ice for forty-eight hours when it is to be used in frequent small transfusions.

#### TIME, QUANTITY AND METHOD OF TRANSFUSION

In our cases both direct and indirect methods of transfusion have been used satisfactorily, although we consider the direct method preferable. In cases where indirect transfusion was given citrated or defibrinated blood was used. A transfusion of 300 to 500 c.c. is usually given every twenty-four to forty-eight hours. In severe rapidly progressive infections small transfusions (100 to 250 c.c.), at intervals of six to twelve hours are of value. In cases where there are toxic myocardial changes or overloading of the pulmonary circulation, as in pneumonia, overburdening of the circulation with added fluids is undesirable, and small transfusions are indicated.

When hemograms are used to study the progress of the infection the transfusion is repeated whenever the left shift and increasing Schilling index indicate that the patient is again beginning to lose ground. Transfusions are repeated daily until the shift is forced right to a Schilling index of less than one.

\* 1/20 of 1 c.c. of commercial typhoid, paratyphoid antigen made up in a one to one billion suspension.

CHART I—Cont'd

CLINICAL				BACTERIOLOGY		HEMOGRAPHY						N S I TRANS.				CAUSE DEATH		PATHOLOGY			
NUMBER OF CASE	COLOR	SEX	AGE	CLINICAL DIAGNOSIS	BLOOD CULTURE: POS. +; NEG. -; NONE 0	BACTERIA	HEMOGRAMMIC DIAGNOSIS, SEP- TICEMIA, S; PYEMIA, P; INFECTIONS, I	HEM. PROG. BEFORE N S I TRANSFUSION; BAD, B	HEM. PROG. 24 TO 48 HR. AFTER LAST N S I TRANS- FUSION, GOOD, G; BAD, B	ADEQUACY OF TREATMENT ESTIMATED BY HEMO- GRAMS, RIGHT SHIFT, IN- PROVEMENT, R; LEFT SHIFT, RETROGRESSION, L	G	L	AVERAGE NUMBER OF MUL- TIPLES GAINED OR LOST PER TRANSFUSION, GAINED, G; LOST, L		NUMBER OF DAYS ILL BEFORE FIRST N S I TRANSFUSION	INTERVALS BETWEEN TRANS- FUSIONS, DAYS, D; HR., H	TOTAL NUMBER OF N S I TRANSFUSIONS	AVERAGE NUMBER OF C.C. PER TRANSFUSION	RESULTS: RECOVERED, R; IMPROVED, I; DIED, D	1. OVERWHELMING INFECTION 2. INEFFECTIVE DRAINAGE 3. INOPERABLE CONDITION, OR 4. INSUFFICIENT NUMBER OF DONORS AVAILABLE	AUTOPSY FINDINGS
15	b	f	20	Metritis, perito- nitis	-		S	B	G	R	25	1	3	2D	2	175	R				
16	w	m	27	Periarthritis nodosa	1		I	B	G	R	2	2	70	3D	4	250	I				
17	w	f	14	Pneumonia, lobar	+	Pneumo. Tp I	S	B	G	R	24		15	3D	2	250	R				
18	w	m	30	Pneumonia, lobar	-		S	B	G	R	23		7	-	1	550	R				
19	w	m	18	Pneumonia, lobar	-		S	B	G	R	26		6	7H	9	169	R				
20	w	f	6	Pneumonia, lobar	-		S	B	G	R	14		4	8H	4	106	R				
21	b	m	45	Pneumonia, lobar	-		S	B	G	R	35		6	1D	2	240	R				
22	w	f	7	Pneumonia, lobar	-		S	B	G	R	6		7	-	1	100	R				
23	w	f	21	Pneumonia, lobar	-		S	B	G	R	100		8	-	1	300	R				
24	w	f	11	Pneumonia, lobar	0		S	B	G	R	13		6	12H	3	75	R				
25	w	m	20	Pneumonia, lobar	-		S	B	G	R	3		11	7D	3	300	R				
26	w	m	38	Abscess, sub- hepatic	+	Strep. hem.	S	B	B	R	50		31	12D	2	450	D	3	Visceral abscesses		
27	w	m	15	Appendicitis, rup. peritonitis	-		S	B	B	R	19		29	-	1	350	D	1 2	Diffuse peritonitis		
28	w	f	13	Agranulocytosis, symptomatic	-		S	B	B	0	0		80	8D	3	500	D	3	Acute reticuloendotheli- osis		
29	b	m	19	Agranulocytosis, symptomatic	+	Staph. albus	S	B	B	R	10		48	2D	3	400	D	1	Aplastic anemia		
30	w	m	56	Agranulocytosis, symptomatic	-		S	B	B	L	16		3	2D	2	250	D	3	Alaemic myelosis		

Chart 1. Showing data on 52 cases treated with hemography-controlled nonspecific immunotransfusion, including the clinical diagnosis; result of blood culture; hemogramic diagnosis before culture was taken; hemogramic prognosis before nonspecific immunotransfusion; hemogramic prognosis twenty-four to forty-eight hours after the last nonspecific immunotransfusion; adequacy of treatment estimated by the hemogramic right or left shift and the gain or loss of multiples per transfusion; number of days ill before transfusion; intervals between transfusions; average number of cubic centimeters per transfusion; final results of treatment; cause of death or apparent failure of nonspecific immunotransfusion to effect a cure and postmortem findings in those cases which were autopsied.

CLINICAL				BACTERIOLOGY	HEMOGRAPHY				N S I TRANS.				CAUSE DEATH		PATHOLOGY				
NUMBER OF CASE	COLOR	SEX	AGE	CLINICAL DIAGNOSIS	BLOOD CULTURE: POS. +; NEG. -; NONE 0	BACTERIA	HEMOGRAMMIC DIAGNOSIS, SEP- TICEMIA, S.; PYEMIA, P.; INFECTIONS, I	HEM. PROG. BEFORE N S I TRANSFUSION; BAD, B	HEM. PROG. 21 TO 48 HR. AFTER LAST N S I TRANS- FUSION; GOOD, G; BAD, B	ADEQUACY OF TREATMENT ESTIMATED BY HEMO- GRAMS, RIGHT SHIFT, IN- PROXIMATE, R; LEFT SHIFT, L; RETROGRADATION, L	AVERAGE NUMBER OF MVL. TITLES GAINED OR LOST PER TRANSFUSION, GAINED, G; LOST, L		NUMBER OF DAYS ILL BEFORE FIRST N S I TRANSFUSION	INTERVALS BETWEEN TRANS- FUSIONS, DAYS, D; HR., H	TOTAL NUMBER OF N S I TRANSFUSIONS	AVERAGE NUMBER OF C.C. PER TRANSFUSION	RESULTS: RECOVERED, R; IMPROVED, I; DIED, D	1. OVERWHELMING INFECTION 2. INFECTIVE DRAINAGE 3. INOPERABLE CONDITION, OR 4. INCURABLE 5. INSUFFICIENT NUMBER OF DONORS AVAILABLE	AUTOPSY FINDINGS
											0	1							
1	b	f	32	Abscesses, empy- ema	-		P	B	G	R		5	5	5	-	1	300	R	
2	b	f	35	Abscesses, teeth	+	<i>Strep. hem.</i>	S	B	G	R		59	40	7D	5	250	R		
3	w	m	35	Appendicitis, rup- peritonitis	-		S	B	G	R		19	2	6H	2	340	R		
4	w	m	35	Appendicitis, rup- peritonitis	-		S	B	G	R		15	2	12H	4	500	R		
5	w	m	14	Arthritis, sup- purative	-		P	B	G	R		14	30	9D	6	206	R		
6	w	m	25	Arthritis, sup- purative	+	<i>Strep. hem.</i>	S	B	G	R		51	22	-	1	300	R		
7	w	m	25	Cellulitis, pec- toral	-		S	B	G	R		33	17	-	1	300	R		
8	w	f	25	Cryptogenic sep- ticemia	+	<i>Strep. hem.</i>	S	B	G	R		34	1	1D	5	300	R		
9	w	f	49	Cryptogenic sep- ticemia	-		S	B	G	R		27	20	2D	11	200	R		
10	w	m	13	Furunculosis	-		S	B	G	R		12	38	5D	3	178	R		
11	w	m	26	Mastoiditis	+	<i>Strep. hem.</i>	P	B	G	R		4	50	12D	5	300	R		
12	w	m	24	Mastoiditis	+	<i>Strep. hem.</i>	S	B	G	R		12	14	5D	2	300	R		
13	w	f	35	Metritis, perito- nitis	-		S	B	G	R		32	18	1D	2	275	R		
14	w	f	34	Metritis, perito- nitis	+	<i>Staph. albus</i>	S	B	G	R		60	17	5D	2	275	R		

CHART I—Cont'd

CLINICAL				BACTERIOLOGY		HEMOGRAPHY										N S I TRANS.				CAUSE DEATH				PATHOLOGY		
NUMBER OF CASE	COLOR	SEX	AGE	CLINICAL DIAGNOSIS	BLOOD CULTURE: POS. +; NEG. -; NONE 0	BACTERIA	HEMOGRAMMIC DIAGNOSIS, SEP- TICEMIA, S.; PYEMIA, P.; INFECTION, I		HEM. PROG. BEFORE N S I TRANSFUSION: BAD, B		HEM. PROG. 24 TO 48 HR. AFTER LAST N S I TRANS- FUSION. GOOD, G; BAD, B		ADEQUACY OF TREATMENT ESTIMATED BY HEMO- GRAMS, RIGHT SHIFT, IM- PROVEMENT, R; LEFT SHIFT, L RETROGRESSION, L		AVERAGE NUMBER OF MUL- TIPLES GAINED OR LOST PER TRANSFUSION, GAINED, G; LOST, L		NUMBER OF DAYS ILL BEFORE FIRST N S I TRANSFUSION	INTERVALS BETWEEN TRANS- FUSIONS. DAYS, D; HR., H	TOTAL NUMBER OF N S I TRANSFUSIONS	AVERAGE NUMBER OF C.C. PER TRANSFUSION	RESULTS: RECOVERED, R; IMPROVED, I; DIED, D	1. OVERWHELMING INFECTION 2. INEFFECTIVE DRAINAGE 3. INOPERABLE CONDITION, OR 4. INSUFFICIENT NUMBER OF DONORS AVAILABLE				AUTOPSY FINDINGS
							I	S	B	B	R	L	G	L	1	2						3	4			
43	W	F	19	Pneumonia, lo- bar, uremia	+	Staph. albus	I	B	B	R	14	6	-	1	1	300	D	1	2	1	2	1	1	Bronchopneumonia, empyema, uremia No autopsy		
44	W	M	49	Pneumonia, lobar	+	Pneumo. Tp. IV	S	B	B	R	72	8	-	1	1	350	D	1	2	1	2	1	1	Lobar pneumonia, supp. pleurisy		
45	W	M	36	Pneumonia, lobar	+	Pneumo. Tp. IV	S	B	B	B	L	40	5	-	1	1	210	D	1	2	1	2	1	1	Lobar pneumonia, cardi- ac decompensation No autopsy	
46	B	F	45	Pneumonia, lobar	-	Pneumo. Tp. IV	S	B	B	R	71	6	-	1	1	400	D	1	3	4	1	3	4	1	Lobar pneumonia	
47	B	M	32	Pneumonia, lo- bar, uremia	-	Pneumo. Tp. IV	S	B	B	B	L	5	25	-	1	1	400	D	1	1	1	1	1	1	Bronchopneumonia, lung abscesses	
48	W	F	40	Pneumonia, lobar	+	Pneumo. Tp. IV Staph. aureus	S	B	B	R	10	14	5D	6	6	200	D	3	3	3	3	3	3	Bronchopneumonia, lung abscesses		
49	W	M	12	Pneumonia, broncho	-	Staph. aureus	S	B	B	R	4	21	-	1	1	180	D	1	3	3	3	3	3	3	Bronchopneumonia, lung abscesses	
50	W	F	17	Pneumonia, broncho	-		S	B	B	R	23	13	1D	6	6	160	D	3	3	3	3	3	3	3	Subac-bact-endocarditis, abscesses brain-kidney	
51	W	F	20	Subacute bact. endocarditis	-		S	B	B	R	13	16	-	1	1	250	D	1	2	4	1	2	4	1	Diffuse peritonitis	
52	W	M	44	Ulcer, perforated duodenal	-		S	B	B	B	L	13	16	-	1	1	250	D	1	2	4	1	2	4	1	Diffuse peritonitis



CHART I—Cont'd

NUMBER OF CASE	COLOR	SEX	AGE	CLINICAL DIAGNOSIS	BACTERIOLOGY			HEMOGRAPHY					N S I TRANS.				CAUSE DEATH		PATHOLOGY
					BLOOD CULTURE: POS. + NEG. - : NONE 0	BACTERIA		HEMORRAGIC DIAGNOSIS. SEP- TICEMIA, S. PYEMIA, P. INFECTIONS, I	HEM. PROG. BEFORE N S I TRANSFUSION: BAD, B	HEM. PROG. 24 TO 48 HR. AFTER LAST N S I TRANS- FUSION. GOOD, G; BAD, B	ADAPQUAY OF TREATMENT ESTIMATED BY HEMO- GRAMS. RIGHT SHIFT, IN- PROVEMENT, R; LEFT SHIFT, L	AVERAGE NUMBER OF MUL- TIPLES GAINED OR LOST PER TRANSFUSION. GAINED, G; LOST, L	NUMBER OF DAYS ILL BEFORE FIRST N S I TRANSFUSION	INTERVALS BETWEEN TRANS- FUSIONS. DAYS, D; HR., H	TOTAL NUMBER OF N S I TRANSFUSIONS	AVERAGE NUMBER OF G.C. PER TRANSFUSION	RESULTS: RECOVERED, R; IMPROVED, I; DIED, D	1. OVERWHELMING INFECTION 2. INOPERABLE CONDITION, OR 3. INCURABLE 4. INSUFFICIENT NUMBER OF DONORS AVAILABLE	
31	w	m	56	Arthritis, sup- purative	+	<i>Strep. hem.</i>	S	B	B	G	R	83	90	54D	2	430	D	1. 3	No autopsy
32	w	f	52	Cellulitis	+	<i>Staph. albus</i>	S	B	B	G	R	5	52	4D	4	170	D	3	Diabetes mellitus, pye- mia, tuberculous brain
33	w	f	72	Gangrene, foot, diabetic	+	<i>Staph. albus</i>	S	B	B	B	R	12	8	-	1	200	D	1	No autopsy
34	w	f	4	Lymphoblastoma, gut obstruc- tion	-		S	B	B	B	L	25	7	-	1	120	D	1	No autopsy, biopsy lymphoblastoma gut
35	w	f	11	Mastoiditis	-		S	B	B	B	R	23	13	1D	6	160	D	1	Mastoiditis, lateral sinus thrombosis
36	w	m	42	Mastoiditis	-		P	B	B	B	R	14	33	1D	5	300	D	2	No autopsy
37	w	f	21	Metritis, perito- nitis	+	<i>Strep. hem.</i>	S	B	B	B	R	8	13	-	1	400	D	2	No autopsy
38	b	f	32	Metritis, perito- nitis	-		S	B	B	B	L	50	4	-	1	500	D	1	No autopsy
39	w	f	27	Metritis, perito- nitis	-		S	B	B	B	R	15	32	1D	2	175	D	1	Metritis, diffuse perito- nitis
40	w	f	30	Metritis, perito- nitis	+	<i>Strep. hem.</i>	S	B	B	B	L	5	2	4D	2	350	D	1	Metritis, diffuse perito- nitis
41	w	f	35	Pneumonia, lobar	-		S	B	B	B	R	18	15	-	1	300	D	1	Bronchopneumonia, ad- enocarcinoma ileum
42	b	m	29	Pneumonia, lo- bar, uremia	+	<i>Staph. albus</i>	I	B	B	B	R	12	37	-	1	460	D	1	No autopsy

Space will permit only a few brief case reports to show variations in types of disease treated and to illustrate the use of the shiftogram.

CASE 8.—(Chart 2.) E. S., a white girl, twenty-five years of age, with a diagnosis of hemolytic streptococci septicemia. The patient ran a severe septic course and eventually lapsed into stupor. Not until then was she given the first of five nonspecific immunotransfusions. These were of 300 c.c. each, given over a period of seven days. Each transfusion was given at the peak of a left shift, and each successively forced the shift right, with a

Date	Hb	Hemoglobin	Mls RBC	Total WBC	MI	Shi	Non-spec. Im.Trans.	Myelocytes	Juveniles	Stabs	Segmenters	Total Polys	Lymphoblasts	Lb	L	I	S	TL	M	E	B	Shftd	Mpls	Hrs	Multiples
561	12	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31
12	60	2.3	19,300	16	1.00			0	12	29	43	84	2	4	0	8	14	2	0	0					
31			14,100	8	1/2			0	15	10	80	75	0	8	0	12	20	5	0	0					
1/4			17,400	14	9/10			0	5	40	50	95	0	2	0	2	4	1	0	0					
6			12,500	5	1/3			0	0	20	40	80	0	10	0	0	10	10	0	0					
11			15,000	21	1.30			0	3	45	36	84	0	8	2	5	15	1	0	0					
13			19,400	48	3.00	1		0	0	60	20	80	0	4	12	4	20	0	0	0					
14			13,300	32	2.00			0	0	60	30	90	0	6	2	0	8	2	0	0					
15			14,000	16	1.00			0	0	40	43	80	2	10	6	0	18	2	0	0	Right	32	36		
15			24,000	88	5.50	2		2	20	60	15	97	0	2	0	1	3	0	0	0	Left	72	12	40	
18			15,600	26	1.60			0	0	50	30	80	0	8	0	4	12	8	0	0					
19	60	2.6	19,800	16	1.00	3		0	4	40	44	88	0	6	0	2	8	4	0	0	Right	72	72	72	
20			15,100	16	1.00			1	6	33	40	80	0	3	0	13	16	4	0	0					
26			10,500	10	2/3			0	2	20	36	58	0	6	2	32	40	2	0	0	Right	6	168		
29	75	3.3	13,200	32	2.00			0	4	36	20	60	0	32	4	2	38	2	0	0					
30			15,300	144	9.00	4		0	4	36	10	98	0	2	0	0	2	0	0	0	Left	134	96	128	
31	75	3.3	13,600	8	1/2			3	3	20	40	68	0	0	10	20	30	2	0	0	Right	136	48		
2			12,600	10	5/8			3	3	20	40	68	0	0	10	20	30	2	0	0					
3			8,800	8	1/2			0	0	24	26	70	0	0	2	28	30	0	0	0					
6			7,400	16	1.00			0	0	32	28	60	0	0	8	32	40	0	0	0					
8			10,200	21	1.30			0	0	38	28	66	0	0	4	30	34	0	0	0					
9			9,200	19	1.20			6	6	27	30	66	0	4	10	20	34	0	0	0					
10			6,200	56	3.50	5		0	8	34	12	54	0	0	0	44	44	2	0	0	Left	48	216	88	
11			12,100	48	3.00			0	1	30	17	68	0	16	1	11	28	2	1	1					
13			11,100	8	1/2			0	2	16	32	50	0	0	14	36	50	0	0	0	Right	48	72		
15			9,800	8	1/2			0	6	16	48	70	0	2	2	16	20	8	2	0					
16			10,100	8	1/2			0	10	10	45	66	0	6	10	18	34	0	0	0					
17			7,500	7	3/7			0	8	10	42	60	0	4	0	34	38	2	0	0					
20			9,800	11	2/3	6*		0	8	12	32	52	0	36	0	8	44	4	0	0	Left	3	144	45	
22			9,800	6	2/5			0	2	22	40	84	0	0	0	16	16	0	0	0					
23			13,400	21	1.30			0	6	36	42	74	0	0	0	26	26	0	0	0					
25			7,300	8	1/2			0	0	18	36	54	0	2	2	42	46	0	0	0					
26			8,900	8	1/2			0	2	14	34	50	0	6	8	36	50	0	0	0					
29			11,300	6	2/5			0	0	16	42	58	0	4	2	36	42	0	0	0					
31			7,000	8	1/2			0	0	18	40	58	0	6	4	30	40	2	0	0					
2			7,800	12	3/4			0	0	30	42	72	0	5	10	13	28	0	0	0					
10			7,600	12	3/4			0	0	30	58	68	0	0	4	28	32	0	0	0					
31/6			8,800	8	1/2			0	0	14	29	42	0	4	0	54	58	0	0	0					
22	85	3.6	6,100	6	2/5			0	4	12	44	60	0	0	0	40	40	0	0	0	Right to recovery				

\* Specific immuno-trans.

\* Specific immuno-trans.

Chart 3.—(Case 2 on Chart 1) B. Y., hemolytic streptococci septicemia (abscessed teeth). Blood stream infection eliminated after five nonspecific immunotransfusions. One specific immunotransfusion was given as the donor had been prepared although the low multiple and Schilling indices did not indicate that it was necessary. Recovery.

corresponding clinical improvement. After the fifth transfusion elimination of the infection and active immunity had developed to a point where the patient was independent of further nonspecific immunotransfusions and continued to complete recovery in nineteen days.

CASE 2.—(Chart 3.) B. Y., a colored woman thirty-five years of age, with a diagnosis of hemolytic streptococci septicemia due to abscessed teeth, was given 1,250 c.c. of blood within twenty-seven days in five nonspecific immunotransfusions of approximately 250 c.c. each. The shift was forced further to the right following each transfusion until it remained within convalescent limits. Ten days later a specific immunotransfusion of 250 c.c. was given as an additional precaution although not indicated by the patient's condition. The infected teeth were removed at intervals of two or three weeks. With the exception of a

The average number of days of illness that elapsed before treatment was 17 in those recovering, and 23 in those dying.

Many of the patients who failed to recover were in apparent need of more than one transfusion, but additional donors were not to be had. This may have reduced the possibility of success of this form of therapy.

Date	Hemoglobin	Mils RBC	Total WBC	Multiple Index	Schilling Index	Non-spec Im. Trans	L. Lymphocytes	C. Juveniles	St. Stabs	Segmentors	Total Polys	Total Lymphs	Lymphoblasts	Large Lympho	I Intermediate Lympho	Small Lympho	Total Lympho	Monocytes	Eosinophiles	Basophiles	Shifted	Multiples	Mrs. Lost	Gained
Shi	D	Hb	RBC	WBC	MI	Shi	Tr	L	C	St	Seg	TH	Lb	L	I	S	T	M	E	B				
3																								
27	85	5.0	15,800	48	3.0			6	18	24	96	0	4	0	0	4	0	0	0					
30			21,200	138	4.6	1		16	20	10	96	0	2	0	0	4	0	0	0					
31			16,100	45	2.8			0	24	25	96	0	1	1	1	3	0	0	1	Right	93	5		
31	85	4.8	19,200	141	8.8	2		22	65	10	98	0	0	1	1	2	0	0	0	Left	96	12	3	
4/1			12,500	22	1.4			0	1	35	85	3	1	3	3	10	4	0	1	Right	119	11		
1			13,700	38	2.4			0	0	25	87	1	3	4	3	11	2	0	0					
2			17,000	48	3.0			0	0	55	20	80	2	7	5	16	4	0	0					
2	80	4.5	17,000	64	4.0	3		4	50	16	80	2	2	10	1	3	16	4	0	Left	42	36		77
3			10,900	12	5/7			0	5	20	87	62	2	20	4	10	36	3	0					
3			22,000	10	3/3	4		0	0	15	45	40	4	34	12	7	57	2	0					
4			18,800	8	1/2			0	0	25	77	2	4	7	8	21	1	1	0	Right	56	36		
4			16,300	16	1.0			0	3	33	64	70	3	12	8	4	27	3	0					
5			15,100	40	2.5			2	4	24	20	70	1	9	8	11	29	0	0	Left	35	36		21
5			15,600	43	2.7	5		2	5	43	22	82	1	2	3	10	16	2	0	Right	29	12		29
6			15,800	14	4/5			0	2	30	39	70	3	15	2	5	25	5	0					
7			17,000	12	3/4			0	2	22	32	56	0	24	11	8	43	1	0					
8			14,500	9	4/7			0	0	20	36	56	4	18	16	0	38	6	0					
8			14,000	9	4/7			0	0	28	48	76	0	0	8	10	18	6	0					
9			12,600	12	3/4			0	0	30	40	70	16	10	0	2	28	2	0					
11	80	4.0	9,200	8	1/2			0	0	10	24	34	0	4	0	62	66	0	0					
12			11,500	6	3/8			0	4	14	43	66	0	6	8	18	32	0	0					
13			7,900	4	1/4			0	4	12	61	78	0	4	8	4	16	4	2					
14			9,400	-3	1/54			0	0	1	59	55	0	2	4	38	44	0	0					
15			10,100	4	1/4			0	0	14	61	76	0	2	0	22	24	0	0					
16			9,400	15	1/1			0	0	4	44	48	0	12	0	40	52	0	0	Right Cont. to recovery				

Chart 2.—(Case 8 on Chart 1.) E. S., hemolytic streptococcal septicemia. By March 30, 1932, the bacterial toxin had forced the shift to the left to a degree of toxicity 138 times greater than normal or a multiple index of 138. At that point the first nonspecific immunotransfusion was given which, within a period of five hours, forced the shift back to the right from a multiple index of 138 to 45 for a gross gain of 93 multiples. Sterilization of the blood stream was not complete but the treatment was hopeful because a temporary right shift had been induced. The bacterial increase during the next twelve hours forced the shift left again from a multiple index of 45 to 141, or a gross loss of 96 multiples. The right shift of 93 multiples induced by the opsonins compared with the left shift 96 multiples induced by the toxin showed a net loss of 3 multiples to the advantage of the infection.

Late March 31, 1932, the second nonspecific immunotransfusion forced the shift right from a multiple index of 141 to 22 for a gross gain of 119 multiples in eleven hours. During the next thirty-six hours the infection forced the shift left from a multiple index of 22 to 64 for a gross loss of 42 multiples. The gain of 119 multiples, by the opsonins, compared with the loss of 42 multiples, by the toxin, showed a net gain of the difference or 77 multiples to the advantage of the nonspecific immunotransfusion.

On Apr. 2, 1932, the third transfusion forced the shift right and the fourth was given Apr. 3, 1932, before the toxin could produce another left shift, so that transfusions 3 and 4 together forced the shift right, in thirty-six hours, from a multiple index of 64 to 8 for a gross gain of 56 multiples. During the following thirty-six hours the infection forced the shift left from a multiple index of 8 to 43 for a gross loss of 35 multiples. This conflict between the opsonins in transfusions 3 and 4 on the one hand, and the infection on the other resulted in a net gain of the difference between 56 and 35 or 21 multiples to the advantage of the nonspecific immunotransfusions.

The fifth and final nonspecific immunotransfusion was given Apr. 5, 1932, and forced the shift right from a multiple index of 43 to 14, in twelve hours, for a net gain of 29 multiples. This was a net gain for the transfusion as no subsequent left shift occurred. The infection was eliminated. The right shift continued during the next ten days, without the aid of further nonspecific immunotransfusions, to a multiple index of 1.5 and to complete recovery of the patient.

An increase in the size of the figure representing the multiple index we speak of as a "loss of multiples" even though it is an increase in the multiple index because it occurs with a left shift, indicates an increase in toxicity, is unfavorable to the patient and extends away from the normal multiple index 1. A decrease in the multiple index is called a "gain of multiples" as it occurs with a right shift, lessened toxicity, is favorable to the patient and approaches the normal multiple index 1.

been running a septic course. A hemogramic diagnosis of pyemia and visceral abscess was made. Blood cultures were negative. Two nonspecific immunotransfusions of 450 c.c. each were given with a resulting shift to the right and marked clinical improvement. Laparotomy then disclosed a large subhepatic abscess. Adequate drainage was impossible; no more transfusions were given and the patient died in forty-eight hours. The final hemogram showed a leucemoid left shift, a Schilling index of 5, a multiple index of 80 and a typical hemogramic picture of septicemia.

CASE 50.—H. L., a white girl seventeen years of age with a streptococci pneumonia, negative blood culture. She was given approximately 2,000 c.c. of blood in nine small transfusions within ten days. The third was a nonspecific immunotransfusion; all others were ordinary transfusions. The shift alternated right and left, terminating in a left shift and the death of the patient after twelve days. One nonspecific immunotransfusion was insufficient and eight ordinary transfusions were of no value in this case.

CASE 11.—T. M., a white male, twenty-six years of age, developed streptococci septicaemia, positive blood culture, three weeks after mastoidectomy. This was followed by pyemia,

Date	Hb	Hemoglobin	Mils RBC	Total RBC	Multiple index	Schilling index	Non-spec Im. Transf.	Myelocytes	Juveniles	Stabs	Segmenters	Total polys	Lymphoblasts	L large lymphocytes	I intermediate lymphs	S small lymphs	Total lymphs	M Monocytes	E Eosinophiles	B Basophiles	Shift	Mpls	Hrs
31	31	32	30	37	22	21	11	12	13	9	6	2	3	1	2	1	2	1	2	1			
14				16,700	32	2.0	1*	0	4	64	32	90	0	5	0	5	10	0	0	0			
15				12,100	48	3.0		2	22	40	22	86	0	8	0	5	14	0	0	0			
16				10,800	48	3.0		0	10	5	23	86	0	6	4	3	13	1	0	0	Left	26	48
17	90	4.6		13,100	40	2.5		2	20	40	26	90	0	6	0	4	10	0	0	0			
18				10,400	21	1.3		0	6	42	36	84	0	8	0	2	10	6	0	0			
19	90	4.6		11,500	32	2.0	2	0	2	50	28	80	0	0	2	18	20	0	0	0	Right	27	48
21				12,100	13	9/11		0	8	26	44	80	0	2	0	16	18	0	0	0	Left	11	24
22				11,900	16	1.0		2	4	34	40	80	0	0	0	20	20	0	0	0	Right	19	48
23				14,000	16	1.0		0	0	38	36	74	0	16	4	6	32	0	0	0			
24				12,700	19	1.2	3	0	0	43	36	78	0	0	0	20	20	0	0	2	Left	6	72
25				10,000	18	1.1		0	8	38	38	84	0	6	2	8	16	0	0	0			
26				8,300	10	3/5		4	6	16	44	70	0	0	10	16	26	4	0	0			
30				7,200	11	7/10		2	6	20	40	68	2	4	4	20	30	0	0	0			
4				9,500	6	2/5		0	0	20	50	70	0	0	0	30	30	0	0	0	Right	13	240
																					Ordinary transfusion.		Recovery

Chart 6.—(Case 12 on Chart 1) C. W., hemolytic streptococci septicaemia, mastoiditis, blood culture positive. The ordinary blood transfusions, one before the shiftogram was begun, and 2 nonspecific immunotransfusions forced the shift right to a convalescent hemogram and recovery in three weeks.

with the appearance of five abscesses which were incised and drained. The patient received seven transfusions of approximately 200 c.c. each, within a period of three weeks. The first was an ordinary transfusion; the following six were nonspecific immunotransfusions. Transfusions and adequate surgical drainage of the abscesses brought about complete recovery in five weeks.

CASE 10.—White male of thirteen years, was admitted to the hospital with a history of furunculosis and a recent tooth extraction, following which he became pale and weak. For a period of four weeks the patient was treated symptomatically, during which time he ran a continuous septic temperature, with repeated negative blood cultures and did not improve. He was then given 500 c.c. of blood in three small nonspecific immunotransfusions, about a week apart. The patient's temperature and hemogram were normal following the third transfusion and he went on to complete recovery.

CASE 17.—C. M., a white girl fourteen years of age, with lobar pneumonia and pneumococemia (Type 1) was given two non-specific immunotransfusions five days apart. Each forced the shift strongly to the right and the temperature fell to normal. The second transfusion kept the shift within convalescent limits and the patient recovered in twelve days.



available. However, the clinical progress of the patient alone may be used as a guide to the use of the nonspecific immunotransfusion. In general, we have noted that the temperature curve of the septic case parallels the shift, a fact noted by Schilling<sup>4</sup> in his original work.

#### CONCLUSIONS

1. Hemography is the most accurate method of following the progress of sepsis and controlling the use of the nonspecific immunotransfusion.

2. The nonspecific immunotransfusion can be used successfully as a helpful therapeutic measure in the treatment of septicemia and other acute infections.

3. The nonspecific immunotransfusion should be used as early as possible in all severe septic conditions. It seems to abort pneumonia. It is not, however, contraindicated in the late stages of disease where it seems almost certain that the patient will die.

4. Of 52 patients treated 25 recovered and 27 died. Five reasons appear to account for failure of recovery: treatment too long delayed, overwhelming infection, insufficient number of donors available, impossibility of adequate drainage, and extensive pathology of incurable character.

We express our thanks to J. A. Fitzmaurice, M.D., Philadelphia, and the Chiefs of the various wards for cooperation and permission to use their cases.

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CASE 12.—(Chart 6.) C. W., a white male of twenty-four years. Following a mastoidectomy he developed symptoms of septicemia, with blood culture positive for *Streptococcus hemolyticus*. Ten cubic centimeters of Pregl's iodine was given intravenously and two ordinary transfusions were given without effect. Later, two nonspecific immunotransfusions of 250 c.c. each were given within five days. The shift was forced progressively to the right. The temperature fell to normal and the patient recovered in three weeks.

CASE 4.—(Chart 7.) D. M., a white male thirty-six years of age, a private case of J. A. Fitzmaurice, M.D., of Philadelphia. A ruptured gangrenous appendix was found at operation. Peritonitis set in and the hemogram was that of a typical septicemia. The patient ran an extremely septic course. Four nonspecific immunotransfusions of 500 c.c. each were given. The first three were given at daily intervals, the fourth after a lapse of three days. The hemogram shifted right progressively, reaching convalescent limits following the fourth transfusion. The patient recovered in sixteen days.

D	Date	Hb	Hemoglobin	RBC	Mils RBC	WBC	Total WBC	MI	Multiple Index	ShI	Schilling Index	T	Non-spec Im. Trans.	M	Myelocytes	J	Juveniles	St	Stabs	Sg	Segments	TN	Total polys	Lb	Lymphoblasts	L	Large lymphocytes	I	Intermediate Lymphs	S	Small Lymphs	Tl	Total Lympho	M	Monocytes	E	Eosinophiles	B	Basophiles	Shifts	Mils	Hrs	Multiples Lost	Multiples Gained																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
31	3/1	32	3/2	33	3/3	34	3/4	35	3/5	36	3/6	37	3/7	38	3/8	39	3/9	40	4/0	41	4/1	42	4/2	43	4/3	44	4/4	45	4/5	46	4/6	47	4/7	48	4/8	49	4/9	50	5/0	51	5/1	52	5/2	53	5/3	54	5/4	55	5/5	56	5/6	57	5/7	58	5/8	59	5/9	60	6/0	61	6/1	62	6/2	63	6/3	64	6/4	65	6/5	66	6/6	67	6/7	68	6/8	69	6/9	70	7/0	71	7/1	72	7/2	73	7/3	74	7/4	75	7/5	76	7/6	77	7/7	78	7/8	79	7/9	80	8/0	81	8/1	82	8/2	83	8/3	84	8/4	85	8/5	86	8/6	87	8/7	88	8/8	89	8/9	90	9/0	91	9/1	92	9/2	93	9/3	94	9/4	95	9/5	96	9/6	97	9/7	98	9/8	99	9/9	100	10/0	101	10/1	102	10/2	103	10/3	104	10/4	105	10/5	106	10/6	107	10/7	108	10/8	109	10/9	110	11/0	111	11/1	112	11/2	113	11/3	114	11/4	115	11/5	116	11/6	117	11/7	118	11/8	119	11/9	120	12/0	121	12/1	122	12/2	123	12/3	124	12/4	125	12/5	126	12/6	127	12/7	128	12/8	129	12/9	130	13/0	131	13/1	132	13/2	133	13/3	134	13/4	135	13/5	136	13/6	137	13/7	138	13/8	139	13/9	140	14/0	141	14/1	142	14/2	143	14/3	144	14/4	145	14/5	146	14/6	147	14/7	148	14/8	149	14/9	150	15/0	151	15/1	152	15/2	153	15/3	154	15/4	155	15/5	156	15/6	157	15/7	158	15/8	159	15/9	160	16/0	161	16/1	162	16/2	163	16/3	164	16/4	165	16/5	166	16/6	167	16/7	168	16/8	169	16/9	170	17/0	171	17/1	172	17/2	173	17/3	174	17/4	175	17/5	176	17/6	177	17/7	178	17/8	179	17/9	180	18/0	181	18/1	182	18/2	183	18/3	184	18/4	185	18/5	186	18/6	187	18/7	188	18/8	189	18/9	190	19/0	191	19/1	192	19/2	193	19/3	194	19/4	195	19/5	196	19/6	197	19/7	198	19/8	199	19/9	200	20/0	201	20/1	202	20/2	203	20/3	204	20/4	205	20/5	206	20/6	207	20/7	208	20/8	209	20/9	210	21/0	211	21/1	212	21/2	213	21/3	214	21/4	215	21/5	216	21/6	217	21/7	218	21/8	219	21/9	220	22/0	221	22/1	222	22/2	223	22/3	224	22/4	225	22/5	226	22/6	227	22/7	228	22/8	229	22/9	230	23/0	231	23/1	232	23/2	233	23/3	234	23/4	235	23/5	236	23/6	237	23/7	238	23/8	239	23/9	240	24/0	241	24/1	242	24/2	243	24/3	244	24/4	245	24/5	246	24/6	247	24/7	248	24/8	249	24/9	250	25/0	251	25/1	252	25/2	253	25/3	254	25/4	255	25/5	256	25/6	257	25/7	258	25/8	259	25/9	260	26/0	261	26/1	262	26/2	263	26/3	264	26/4	265	26/5	266	26/6	267	26/7	268	26/8	269	26/9	270	27/0	271	27/1	272	27/2	273	27/3	274	27/4	275	27/5	276	27/6	277	27/7	278	27/8	279	27/9	280	28/0	281	28/1	282	28/2	283	28/3	284	28/4	285	28/5	286	28/6	287	28/7	288	28/8	289	28/9	290	29/0	291	29/1	292	29/2	293	29/3	294	29/4	295	29/5	296	29/6	297	29/7	298	29/8	299	29/9	300	30/0	301	30/1	302	30/2	303	30/3	304	30/4	305	30/5	306	30/6	307	30/7	308	30/8	309	30/9	310	31/0	311	31/1	312	31/2	313	31/3	314	31/4	315	31/5	316	31/6	317	31/7	318	31/8	319	31/9	320	32/0	321	32/1	322	32/2	323	32/3	324	32/4	325	32/5	326	32/6	327	32/7	328	32/8	329	32/9	330	33/0	331	33/1	332	33/2	333	33/3	334	33/4	335	33/5	336	33/6	337	33/7	338	33/8	339	33/9	340	34/0	341	34/1	342	34/2	343	34/3	344	34/4	345	34/5	346	34/6	347	34/7	348	34/8	349	34/9	350	35/0	351	35/1	352	35/2	353	35/3	354	35/4	355	35/5	356	35/6	357	35/7	358	35/8	359	35/9	360	36/0	361	36/1	362	36/2	363	36/3	364	36/4	365	36/5	366	36/6	367	36/7	368	36/8	369	36/9	370	37/0	371	37/1	372	37/2	373	37/3	374	37/4	375	37/5	376	37/6	377	37/7	378	37/8	379	37/9	380	38/0	381	38/1	382	38/2	383	38/3	384	38/4	385	38/5	386	38/6	387	38/7	388	38/8	389	38/9	390	39/0	391	39/1	392	39/2	393	39/3	394	39/4	395	39/5	396	39/6	397	39/7	398	39/8	399	39/9	400	40/0	401	40/1	402	40/2	403	40/3	404	40/4	405	40/5	406	40/6	407	40/7	408	40/8	409	40/9	410	41/0	411	41/1	412	41/2	413	41/3	414	41/4	415	41/5	416	41/6	417	41/7	418	41/8	419	41/9	420	42/0	421	42/1	422	42/2	423	42/3	424	42/4	425	42/5	426	42/6	427	42/7	428	42/8	429	42/9	430	43/0	431	43/1	432	43/2	433	43/3	434	43/4	435	43/5	436	43/6	437	43/7	438	43/8	439	43/9	440	44/0	441	44/1	442	44/2	443	44/3	444	44/4	445	44/5	446	44/6	447	44/7	448	44/8	449	44/9	450	45/0	451	45/1	452	45/2	453	45/3	454	45/4	455	45/5	456	45/6	457	45/7	458	45/8	459	45/9	460	46/0	461	46/1	462	46/2	463	46/3	464	46/4	465	46/5	466	46/6	467	46/7	468	46/8	469	46/9	470	47/0	471	47/1	472	47/2	473	47/3	474	47/4	475	47/5	476	47/6	477	47/7	478	47/8	479	47/9	480	48/0	481	48/1	482	48/2	483	48/3	484	48/4	485	48/5	486	48/6	487	48/7	488	48/8	489	48/9	490	49/0	491	49/1	492	49/2	493	49/3	494	49/4	495	49/5	496	49/6	497	49/7	498	49/8	499	49/9	500	50/0	501	50/1	502	50/2	503	50/3	504	50/4	505	50/5	506	50/6	507	50/7	508	50/8	509	50/9	510	51/0	511	51/1	512	51/2	513	51/3	514	51/4	515	51/5	516	51/6	517	51/7	518	51/8	519	51/9	520	52/0	521	52/1	522	52/2	523	52/3	524	52/4	525	52/5	526	52/6	527	52/7	528	52/8	529	52/9	530	53/0	531	53/1	532	53/2	533	53/3	534	53/4	535	53/5	536	53/6	537	53/7	538	53/8	539	53/9	540	54/0	541	54/1	542	54/2	543	54/3	544	54/4	545	54/5	546	54/6	547	54/7	548	54/8	549	54/9	550	55/0	551	55/1	552	55/2	553	55/3	554	55/4	555	55/5	556	55/6	557	55/7	558	55/8	559	55/9	560	56/0	561	56/1	562	56/2	563	56/3	564	56/4	565	56/5	566	56/6	567	56/7	568	56/8	569	56/9	570	57/0	571	57/1	572	57/2	573	57/3	574	57/4	575	57/5	576	57/6	577	57/7	578	57/8	579	57/9	580	58/0	581	58/1	582	58/2	583	58/3	584	58/4	585	58/5	586	58/6	587	58/7	588	58/8	589	58/9	590	59/0	591	59/1	592	59/2	593	59/3	594	59/4	595	59/5	596	59/6	597	59/7	598	59/8	599	59/9	600	60/0	601	60/1	602	60/2	603	60/3	604	60/4	605	60/5	606	60/6	607	60/7	608	60/8	609	60/9	610	61/0	611	61/1	612	61/2	613	61/3	614	61/4	615	61/5	616	61/6	617	61/7	618	61/8	619	61/9	620	62/0	621	62/1	622	62/2	623	62/3	624	62/4	625	62/5	626	62/6	627	62/7	628	62/8	629	62/9	630	63/0	631	63/1	632	63/2	633	63/3	634	63/4	635	63/5	636	63/6	637	63/7	638	63/8	639	63/9	640	64/0	641	64/1	642	64/2	643	64/3	644	64/4	645	64/5	646	64/6	647	64/7	648	64/8	649	64/9	650	65/0	651	65/1	652	65/2	653	65/3	654	65/4	655	65/5	656	65/6	657	65/7	658	65/8	659	65/9	660	66/0	661	66/1	662	66/2	663	66/3	664	66/4	665	66/5	666	66/6	667	66/7	668	66/8	669	66/9	670	67/0	671	67/1	672	67/2	673	67/3	674	67/4	675	67/5	676	67/6	677	67/7	678	67/8	679	67/9	680	68/0	681	68/1	682	68/2	683	68/3	684	68/4	685	68/5	686	68/6	687	68/7	688	68/8	689	68/9	690	69/0	691	69/1	692	69/2	693	69/3	694	69/4	695	69/5	696	69/6	697	69/7	698	69/8	699	69/9	700	70/0	701	70/1	702	70/2	703	70/3	704	70/4	705	70/5	706	70/6	707	70/7	708	70/8	709	70/9	710	71/0	711	71/1	712	71/2	713	71/3	714	71/4	715	71/5	716	71/6	717	71/7	718	71/8	719	71/9	720	72/0	721	72/1	722	72/2	723	72/3	724	72/4	725	72/5	726	72/6	727	72/7	728	72/8	729	72/9	730	73/0	731	73/1	732	73/2	733	73/3	734	73/4	735	73/5	736	73/6	737	73/7	738	73/8	739	73/9	740	74/0	741	74/1	742	74/2	743	74/3	744	74/4	745	74/5	746	74/6	747	74/7	748	74/8	

Chart 7.—(Case 4 on Chart 1) D. M.G., peritonitis, ruptured appendix, septicemia clinically and by hemogram. Adequate drainage and four nonspecific immunotransfusions given by J. A. Fitzmaurice, M.D., Philadelphia, in his surgical practice, induced right shifts and convalescent hemogram in ten days and recovery in sixteen days.

#### COMMENT

Nonspecific immunotransfusion therapy is based on the principle of increasing the formation of nonspecific antibodies, or opsonins in the blood of the donor by the intravenous injection of killed typhoid organisms. This was first described by Sir Almroth Wright in 1919 and by Colebrook and Storer in 1923.

We have found that the opsonins in the donor's blood are increased from five to ten times eight hours after the intravenous injection of 50,000,000 killed typhoid bacilli.

Clinical evidence supports the theory of increased antibody formation, as a single nonspecific immunotransfusion is sometimes sufficient to sterilize the blood stream, at least temporarily. Moreover, it has been clinically observed that the use of the nonspecific immunotransfusion seems to facilitate resolution or localization of the infective foci.

Hemography was used in these cases as being the best indicator of the course of the infection and the intervals at which transfusions should be repeated. This, in our opinion, is the best plan where daily hemograms are

pressure changes in the enterograph balloon permits a flexible connection between enterograph and recording surface. The enterograph is suspended above its segment from a horizontal rod by means of a long string, and is thus able to move freely with mass shifting of position of the segment. Such an arrangement is of considerable value in recording the large intestine, where the powerful longitudinal contractions of the distal segment may drag the entire colon analward (Lawson and Templeton, 1932). With three enterographs attached longitudinally along the colon, fixed levers toward the anus, it has been possible to obtain strong longitudinal contractions in one segment without change in the records of adjacent segments. The enterographs can be attached longitudinally,

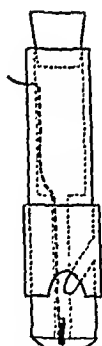


Fig. 3.

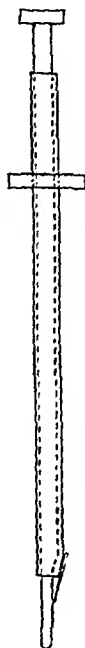


Fig. 4.

Fig. 3.—Fluid electrode. A hard rubber rod 4 cm. long and 1 cm. in cross-sectional diameter is drilled for half its length to form a thin-walled vial. The remaining length of the rod is drilled with two small drills placed side by side to form smaller channels connecting with the vial. (Only one of these is seen in the profile view.) An oblique saw cut opens the two channels about 1 cm. from the bottom of the rod. Enamelled copper wires are led through openings near the top of the vial, and are soldered to silver electrodes which are fixed with cement at the bottom of each channel. A sleeve of hard rubber slips down over the oblique slit, covering all except the lower extremities. A notch in the lower edge of the sleeve may be rotated into position to permit the occlusion of one extremity while the other is left open, so that the electrode may be applied to either the intact nerve, or to the stump of the divided nerve.

Fig. 4.—Neurotome. A steel rod about 3 mm. in diameter is fitted loosely into a length of brass tubing. A small strip of spring steel is soldered obliquely to the lower end of the tubing so as to project about 5 mm. beyond its end. The free edge of the spring steel is sharpened to a knife edge. The inner rod is ground flat on one side to slip past the knife, and is slotted obliquely for holding the nerve. After the nerve is inserted in the slot, the rod is drawn up until its lateral opening is blocked by the knife. The nerve is divided by drawing the rod upward.

circularly, or crossed on the same segment for studying longitudinal-circular relationships. The placement of fulera for the levers is such as to leave an open space between the fixed and the movable lever to facilitate crossing the enterographs in the latter type of recording.

For stimulation of visceral nerves in the fluid-filled belly, two types of shielded electrodes were employed. The earlier type is suitable for occasional



# LABORATORY METHODS

## FURTHER NOTES ON ENTEROGRAPHIC TECHNIC\*

WITH SPECIAL REFERENCE TO THE STUDY OF INNERVATION

HAMPDEN LAWSON, PH.D., LOUISVILLE, KY.

IN 1932 Templeton and Lawson published a description of technic used in recording movements of the large intestine in unanesthetized, trained dogs. It subsequently became necessary to modify some of the reported methods for use in an investigation of the extrinsic nerves of the large intestine in anesthetized animals with the viscera exposed. All the apparatus described can be built in the average modestly equipped shop.

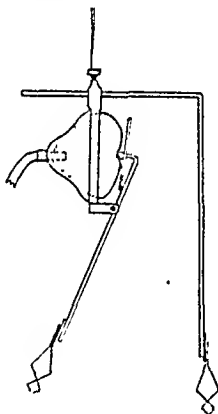


Fig. 1.

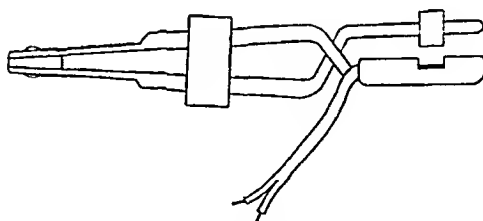


Fig. 2.

Fig. 1.—Enterograph. The bell-shaped receptacle for the recording balloon is made by hammering out a Harvard ring tambour. The fulcrum for the movable lever and the binding post for holding the fixed lever are soldered to the ring across its diameter. A ratio of 3:1 between force and weight arms of the movable lever gives a magnification of approximately three times in the record, with a balloon of 20 c.cm. volume, and a manometer with a bore of 1 cm. The serrefines were made from weaving needles.

Fig. 2.—Shielded metal electrode. The construction is explained in the text.

For recording the muscular activity of the colon, an enterograph was made for attachment by means of serrefines to the outside of the exposed segment. The new feature in this enterograph is the conversion of movements of the levers attached to the gut into pressure changes in a condom balloon, allowing manometric recording of motility (Fig. 1). The conversion of lever movements into

\*From the Department of Physiology and Pharmacology, University of Louisville School of Medicine.

Received for publication, May 11, 1934.

## A GRAVIMETRIC METHOD OF DETERMINING OXYGEN CONSUMPTION IN MAN\*

J. P. HETTWER, PH.D., MILWAUKEE, WIS.

IN TEACHING students the principles of the various methods of measuring gaseous metabolism in man, the laboratory exercise commonly provides for comparison tests with open and closed circuit volumetric apparatus. For a more complete survey and to show fundamental relationships it has often seemed desirable to include a gravimetric measurement of oxygen consumption. This generally requires a special and costly balance for the weighing of a metal cylinder of compressed oxygen. In the apparatus here described the oxygen, at atmospheric pressure in a light, flexible rubber bag, is weighed on an ordinary prescription balance. By properly utilizing the rebreathing method, partitioning valves or electric impeller are obviated. A year's experience with the apparatus seems to indicate that the factors emphasized form convenient starting points for student laboratory discussion and probably stimulate increased interest in the subject of general metabolism.

A diagrammatic sketch of the apparatus is given in Fig. 1. In principle, the subject rebreathes through a small soda lime chamber close to the mouth, the oxygen-rich air in a can for a convenient length of time. Two rubber bags, containing pure oxygen and communicating only with each other, one sealed inside the can and the other exposed outside, together act as expansion chamber in place of a spirometer bell. There are, therefore, two separate gas systems, the can, soda lime chamber and subject on the one hand, the two rubber bags on the other hand and the latter is adequately responsive to any volume change in the former. Before beginning a test most of the oxygen of the bag system is in the outer bag. As oxygen consumption from the can proceeds during a test period the inner bag unfolds and the outer refolds; that is, oxygen from the outer bag gradually passes into the inner bag. The difference between the oxygen content of the outer bag at the beginning and at the end of a test period is a measure of the oxygen consumed or withdrawn from the can. This difference is determined by weighing the outer bag before the beginning and after the end of the test period.

Rebreathing of oxygen or oxygen-rich air from which the carbon dioxide is constantly abstracted may be considered entirely safe for short-period observations if the absorbent chamber is small, of copper construction for high emissivity of heat, filled with low alkali soda lime (Wilson's) and placed very close to the mouth. One of the essential features of Jackson's closed system of

\*From the Department of Physiology, Marquette University School of Medicine.  
Received for publication, May 24, 1934.

stimulations, and is recommended for this purpose by its simplicity. It was constructed by cementing a grooved hard rubber block of the conventional type, with the electrodes exposed at the bottom of the groove, to one jaw of a small bulldog artery clamp. A soft rubber ring cut from tubing, slips over the opposing jaw of the clamp, and holds the nerve against the metal electrodes (Fig. 2). Pressure against the nerve is adjusted by means of a metal ring, moved along the handle, which forces the jaws of the clamp apart. Diffusion is limited by threading the nerve through a pinhole in a large square of condom rubber, which is then tied in the form of a sac about the electrode and contained nerve.

For repeated stimulations over long periods of time, where the preservation of irritability of the nerve was to be insured, fluid electrodes modified from the type described by Brown and Garry (1932) were employed. The modification of their electrode is justified by the greater ease with which the nerve can be inserted, and by the fact that the modified electrode can be used on the intact nerve. The modified electrode is essentially a small hard-rubber vial which the nerve enters through an oblique slit. The slit is then occluded except for its extremities, which are occupied by the nerve, by means of a sleeve which is moved downward over the slit. For use on the divided nerve, the sleeve is rotated so that a notch in its lower edge coincides with the extremity occupied by the entering nerve. On being slipped down, the sleeve now occludes the entire slit except for this opening, and the nerve is anchored within the vial by means of its holding ligature, bound between the sleeve and the wall of the vial (Fig. 3). The electrode is vaseline sealed, filled with saline, and stoppered.

For dividing nerves during the course of the experiment without disturbing the viscera, special neurotomes were constructed. Their principle is that of the cutting ligature. Their advantage lies in the fact that they produce less trauma, and that the division is made much more certain (Fig. 4).

#### REFERENCES

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Lawson, H., and Templeton, R. D.: *Am. J. Physiol.* 100: 362, 1932.  
Templeton, R. D., and Lawson, H.: *J. LAB. & CLIN. MED.* 17: 1244, 1932.

when the humidity of the room air is exceedingly low. The former is adequately eliminated by having doors and windows closed and not making any rapid movements during the actual weighing. The possible error due to electrostatic charge on the bag is minimized to negligible proportions by mounting the balance in such a way that the suspended bag is at least 50 cm. from the nearest massive object such as wall, table, bed, chair, etc., and by the observer himself standing at least the same distance away from the bag while watching the pointer and scale of the balance.

*Technic of an Experiment.*—The procedure in handling the apparatus is the following:

a. After the apparatus is perfectly equilibrated to room temperature on a table next to and of the same height as the bed, the  $\frac{1}{2}$ -inch 3-way valve is opened to the oxygen can. The empty rubber bag is attached to the nipple on the opened shut-off valve. Care should be exercised in handling the can and bag to transmit as little body heat as possible.

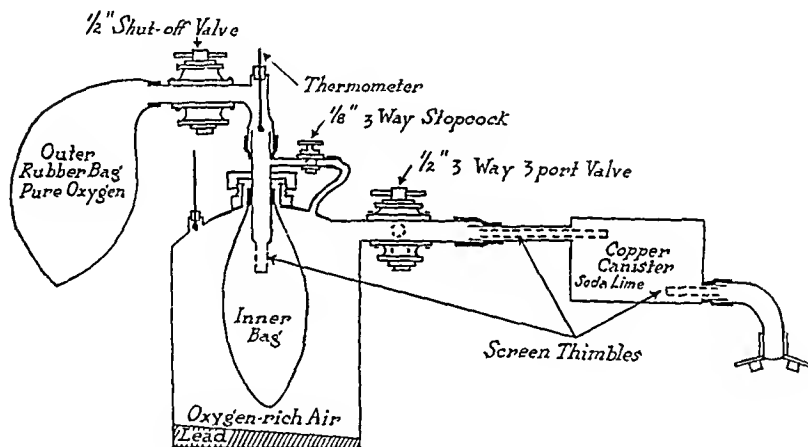


Fig. 1.

b. By means of the 3-way oxygen distribution stopcock, supply oxygen is introduced into the can allowing an escape through the soda lime canister for two or three seconds. The  $\frac{1}{2}$ -inch 3-way valve is then closed to the can and more oxygen allowed to enter, thus deflating the inner bag and unfolding the outer one. When the outer one no longer enlarges, the supply gas is stopped. If the apparatus has not been used for some time, it is well to remove the outer bag at this time, empty and reattach it.

c. The 3-way distribution valve is now turned and supply oxygen allowed to enter the bag system. It will pass only into the outer bag and when that is unfolded to about three-fourths capacity (about four liters) the gas is stopped.

d. The  $\frac{1}{2}$ -inch 3-way valve is then opened to the can momentarily allowing any pressure within to drop to zero. When the outer bag just begins to get smaller the valve is closed to the can again. Also the shut-off valve is now closed.

e. Next, the outer bag is clamped close to the nipple, detached, suspended on the balance placed nearby and weighed to a fineness of 5 mg. At least five

anesthesia (Jackson, 1918) adapted by Waters for human anesthesia (Waters, 1932) is rebreathing under these conditions. Gas analyses by Waters showed that with only 500 gm. of Wilson's soda lime 4-8 mesh, close to the mouth, a patient could rebreathe oxygen from a rubber bag for one-half hour without a trace of carbon dioxide appearing in the bag. To increase the margin of safety and also to have the interspaces between the soda lime granules approximately equal to normal tidal air, the soda lime canister is made to hold about 600 gm.

It will be noted that by having screen thimbles in inlet and outlet tubes, the soda lime canister does not require a screw-cover. The tubes themselves are placed at diametrically opposite sides whereby a simple rotation of the canister provides for about 10 cm. of height adjustment of the mouthpiece in arranging the apparatus to the subject. The two movable junction points are covered by a short piece of  $\frac{3}{4}$ -inch rubber tubing, wired at one end and clamped at the other, after the height adjustment has been made, by an ordinary small radiator hose clamp with wing screw.

The oxygen can has a capacity of about 8 liters so that the inner rubber bag can unfold freely and completely. A two-gallon gasoline can is easily adapted to the purpose. Molten lead covered by a layer of paraffin is poured into the bottom to counterbalance the weight of the soda lime canister and tubing which extend about 50 cm. from the side of the can.

The  $\frac{1}{2}$ -inch, 3-way, 3-port valve serves the usual purpose of such a valve in a closed system, namely, of permitting the subject to breathe outside air for a few minutes before the actual test period. A small feather attached to the side opening facilitates gauging of the right moment to turn the valve. The position of the valve should really be between mouthpiece and soda lime canister. However, reaching out in front of the subject to turn the valve may alter the breathing and so introduce an error. Of course, attachments could be made to operate the valve from a distance.

The two rubber bags are alike holding 6 liters when unfolded but not stretched. Breathing bags made for the Waters' anesthesia apparatus, weighing from 75 to 100 gm. each, serve well enough, although, presumably, lighter and equally durable bags can be manufactured. The neck of the inner bag is wired to the tube passing through the screw-cap into the oxygen can. The neck of the outer bag is slipped or stretched over the chromium plated nipple projecting from the  $\frac{1}{2}$ -inch shut-off valve. An ordinary pinchcock (Day's) serves to close the opening of this bag before it is detached for weighing.

Weighing of the oxygen bag presents no great difficulty. The bearing and beam of an inexpensive student or prescription balance, having a sensitivity of 5 mg. and a capacity of about 100 gm., is removed from its base and suitably mounted by clamps and long T-arm on a laboratory stand. By means of a small loop of wire attached to the pinchcock, the oxygen bag is suspended from the hook at one end of the beam while at the same time a combined scale pan and tare weight is suspended from the hook at the other end of the beam. Only fractional metric weights are needed to complete the weighing.

Two sources of error in weighing require attention. One is due to strong currents of air in the room and the other to electrostatic charge on the bag

gases, the weight of a liter of humid air, that is, its specific gravity,  $S'_e$ , is given by the following:

$$S'_e = S_o \cdot \frac{P - h}{760(1 + 0.00367t)} + 0.624 \cdot \frac{S_o^1 h}{760(1 + 0.00367t)} = S_o^1 \cdot \frac{P - 0.376h}{760(1 + 0.00367t)}$$

where  $h$  is the partial pressure of the water vapor at temperature,  $t$ . If now the proper equivalents of  $S'_e$ ,  $S_E$ ,  $V_E$ ,  $S_o$ , and  $S_o^1$  are substituted in (1), then,

$$V_o = \frac{Pw}{0.136P + 0.486h} \quad (3)$$

which takes into account the humidity of the room air.

For convenience the basic expression (2) is retained and a humidity correction factor,  $f$ , determined so that,

$$V_o = f \cdot \frac{w}{0.136} \quad (4)$$

The way  $P$  appears in expression (3) it will suffice to assume a mean barometric pressure, say 745 mm. Hg, so that on solution:  $f = \frac{1}{1 + 0.0048h}$

Considering the depression of a wet bulb thermometer when whirled to provide a motion of at least three meters per second, as an adequate indication of the humidity and taking the corresponding value of  $h$  from standard psychrometric tables, the humidity factor  $f$  for a probable range of room temperature from 16° to 30° C. has been calculated. These factors comprise the body of Table I.

TABLE I

FACTORS, CORRESPONDING TO ROOM TEMPERATURE AND DEPRESSION OF THE WET BULB THERMOMETER, WHICH WHEN MULTIPLIED BY THE APPARENT WEIGHT IN ROOM AIR OF THE OXYGEN CONSUMED, GIVE ITS APPARENT WEIGHT IN ABSOLUTELY DRY AIR  
DEPRESSION OF WET BULB THERMOMETER  
 $t^\circ - t^\circ \text{ C.}$

DRY BULB $t^\circ \text{ C.}$	1°	2°	3°	4°	5°	6°	7°	8°	9°	10°	11°	12°	13°	14°
16°	0.95	0.95	0.96	0.96	0.97	0.97	0.98	0.98	0.99					
18°	0.94	0.94	0.95	0.95	0.96	0.97	0.97	0.98	0.98	0.99				
20°	0.93	0.94	0.94	0.95	0.95	0.96	0.96	0.97	0.98	0.98	0.99			
22°	0.92	0.93	0.93	0.94	0.95	0.95	0.96	0.96	0.97	0.97	0.98	0.99		
24°	0.91	0.92	0.92	0.93	0.94	0.94	0.95	0.96	0.96	0.97	0.97	0.98	0.98	0.99
26°	0.90	0.91	0.92	0.92	0.93	0.94	0.94	0.95	0.95	0.96	0.97	0.97	0.98	0.98
28°	0.89	0.90	0.90	0.91	0.92	0.93	0.93	0.94	0.95	0.95	0.96	0.97	0.97	0.98
30°	0.88	0.89	0.89	0.90	0.91	0.92	0.92	0.93	0.94	0.94	0.95	0.96	0.96	0.97

The effect of humidity of the room air on the apparent weight of the gas becomes negligible if a gas is chosen having a specific gravity considerably higher than air, e.g., carbon dioxide, or considerably lower, e.g., hydrogen. While it is feasible to use a gas other than oxygen in the bag system of the apparatus, it means the availability and handling of an additional gas. For students and from a teaching standpoint, it is preferable to use oxygen and apply the humidity correction factor.

minutes should be allowed for the weighing. The bag is then reattached to the nipple, the pinchelamp removed and the shut-off valve opened. The apparatus is now ready for the actual test.

f. The rubber mouthpiece is attached to the chromium plated copper elbow and the whole apparatus adjusted to the subject. Having applied the nose-clip, the subject is permitted to breathe through the side opening of the 3-way valve for a minute, or longer if necessary, to assure composure.

g. At the bottom of an apparently normal expiration, the  $\frac{1}{2}$ -inch 3-way valve is quickly turned. At the same time a stop watch is started or the time noted to the second on an ordinary watch.

h. As the test period approaches its end, the movements of the bag are observed together with the watch held near it and at the bottom of the expiration closest to the end of the period, the  $\frac{1}{2}$ -inch 3-way valve is quickly turned again and the shut-off valve closed at once thereafter. If the respirations seem to be somewhat abnormal one may wait for a more favorable moment before ending the test and calculate accordingly.

i. The subject is now freed. The bag is clamped, removed and weighed as before.

*Interpretation and Calculation.*—The following considerations apply. The difference between first and second weighing represents the apparent weight in air of the volume of oxygen withdrawn from the bag in the test period. According to Archimedes' principle applied to gases, the oxygen when weighed in air loses in weight an amount equal to the weight of the volume of air displaced. The real weight of a gas is equal to its volume times its specific gravity. If  $w$  is the apparent weight of the oxygen in air;  $V_E$ , the volume of the oxygen as well as of the displaced air under experimental conditions;  $S_E$ , the specific gravity of the oxygen; and  $S_L^1$ , the specific gravity of the air under experimental conditions, then,  $V_E S_E = w + V_E S_L^1$

$$\text{whence, } V_E = \frac{w}{S_E - S_L^1} \quad (1)$$

The volume of a gas at standard conditions is:  $V_0 = V_E \cdot \frac{P}{760(1 + 0.00367t)}$  and the specific gravity is:  $S_0 = S_E \cdot \frac{760(1 + 0.00367t)}{P}$  where  $P$  and  $t$  are the experimental pressure and temperature respectively. If temperature and pressure are the same for the air as for the oxygen, substitution in (1) yields:

$V_0 = \frac{w}{S_0 - S_L^1}$ . Taking  $S_0$  as 1.429 gm. per liter and  $S_L^1$  as 1.293 gm., the following basic expression is obtained:

$$V_0 = \frac{w}{0.136} \quad (2)$$

However, while temperature and total pressure are the same for the air as for the oxygen, one cannot neglect the partial pressure of water vapor in the air and its effect in decreasing the specific gravity, since the density of water vapor is only 0.624 that of air. From the gas laws concerning the mixture of

safely disregarded, although it is well to know that its direction is also toward too low an estimate.

*Accuracy.*—The alcohol check test (Carpenter, 1915; Carpenter and Fox, 1923) has not been applied; but the common method of testing by comparison with other apparatus of known accuracy on the same human subject has been used repeatedly. Table III gives typical results of comparison with the closed circuit volumetric laboratory apparatus.

TABLE III  
OXYGEN CONSUMPTION PER MINUTE  
SUBJECT, MISS R. M. H.

TIME	APPARATUS	O <sub>2</sub> PER MINUTE
		c.c.
8:45 A.M.	Gravimetric	187
9:30	Gravimetric	177
10:00	Volumetric	189
10:20	Volumetric	179
10:40	Volumetric	177
11:00	Gravimetric	177

Six consecutive tests under basal conditions were made on one subject, Miss R. M. H., who had had some experience on both types of apparatus and whose breathing record on the volumetric was exceptionally even so that the oxygen line could be drawn with confidence. The agreement is as close as can be expected. The students have made many single comparison tests on normal subjects with no greater than 6 per cent disagreement. This is better than anticipated. The greatest satisfaction to the teacher, however, lies in the healthy discussion on fundamentals, provoked by such comparison tests.

Undoubtedly the method has decided limitations. In the nature of it some care must be exercised to secure an adequate constancy of temperature, pressure, and humidity. Moreover, there is no graphic record of respiratory movements and all that that precludes. This is no disadvantage from a teaching standpoint when the apparatus is used by students in comparison testing with graphic volumetric apparatus. As far as accuracy is concerned, it is to be recognized that in either method the degree of expansion of the lungs of the subject is an uncontrolled factor.

It may be mentioned here that the apparatus and gravimetric method can be adapted to serve for the determination of lung volumes, particularly residual air, thereby obviating the usual gas analysis. A report of this method will be given in another paper.

#### SUMMARY

An inexpensive apparatus for the gravimetric determination of oxygen consumption in man, adequate for student laboratory comparison tests with volumetric types, is described. In principle, the subject rebreathes the oxygen-rich air in a can through a small soda lime canister placed close to the mouth. A light flexible rubber bag sealed inside the can communicates with a similar one outside containing oxygen. The two together act as expansion chamber in place of a spirometer bell but form a separate gas system. The difference between



The desired end-result is, therefore, conveniently calculated on the basis of expressions such as the following:

Liters O<sub>2</sub> per test period =  $f \cdot \frac{w}{0.136} = 7.353 \cdot f \cdot w$

e.c. O<sub>2</sub> per minute =  $\frac{735.30f \cdot w}{\text{Time} \begin{smallmatrix} \text{(in minutes and)} \\ \text{decimal fraction)} \end{smallmatrix}}$

Calories per sq. meter per hr. =  $\frac{354.78 \cdot f \cdot w}{\text{Body Surface}}$   
(Basis of six minute test and  
calorific value of O<sub>2</sub> = 4.825)

It is not difficult to prepare a height-weight table in which not the actual body surface is given but 354.78 divided by the surface for each height-weight combination. Or, given the body surface, one may find in Table II, 354.78 divided by the surface which is then simply multiplied by the corrected weight difference. The students are, of course, encouraged to make the calculation step by step in both gravimetric and volumetric methods.

TABLE II  
FACTORS, CORRESPONDING TO BODY SURFACE IN SQ. METERS, WHICH WHEN MULTIPLIED BY THE APPARENT WEIGHT IN AIR OF THE OXYGEN CONSUMED CORRECTED FOR HUMIDITY OF ROOM AIR, GIVE THE CALORIES PER SQ. METER PER HR. ON THE BASIS OF A SIX-MINUTE TEST PERIOD

Factor =  $\frac{4.825 \times 10}{0.136 \times \text{Body Surface}} = \frac{354.78 \cdot f \cdot W}{\text{Body Surface}}$

BODY SURFACE SQ. METERS	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
1.20	296	294	291	289	286	283	281	279	277	275
1.30	273	271	269	267	265	263	261	259	257	255
1.40	253	252	250	248	247	245	244	242	240	239
1.50	237	235	234	232	231	229	228	226	225	223
1.60	222	220	219	218	216	215	214	213	211	210
1.70	209	208	206	205	204	203	202	200	199	198
1.80	197	196	195	194	193	192	191	190	189	188
1.90	187	186	185	184	183	182	181	180	179	178
2.00	177	177	176	175	174	173	172	171	171	170
2.10	169	168	167	167	166	165	164	164	163	162
2.20	161	161	160	159	158	158	157	156	156	155
2.30	154	154	153	152	152	151	150	150	149	148

There are several sources of error that operate in the reverse direction to humidity of the room air, that is, result in too low an estimate of oxygen consumption, if neglected. The method assumes that there is no change of temperature, pressure, or humidity within the closed systems during the test period. While temperature and total pressure do practically remain constant, it is clear that, with only Wilson's soda lime in the canister, there may be a considerable change of water vapor in the can. It is important to minimize this change either by adding a layer of calcium chloride to the soda lime in a larger canister or by keeping a small quantity of sterile water in the bottom of the oxygen can. Neither method is probably completely effective (Roth, 1922); but in any case, the error assumes negligible proportions. Finally, there is the fact that ordinary supply oxygen contains impurities, particularly nitrogen, which make its specific gravity somewhat less than the assumed 1.429 gm. The error may be

## DESCRIPTION OF THE APPARATUS

The objective was to deliver the solution through the needle into the circulation at the normal body temperature and to maintain this temperature at a uniform level throughout the course of the infusion. To this end the apparatus described below was constructed. Briefly, it consisted of a water-bath, the temperature of which was automatically controlled, into which the sterilized intravenous jar could be inserted and removed following the infusion. The apparatus was built on a portable stand (Fig. 3). This principle is believed not to have been described heretofore for the particular purpose involved.

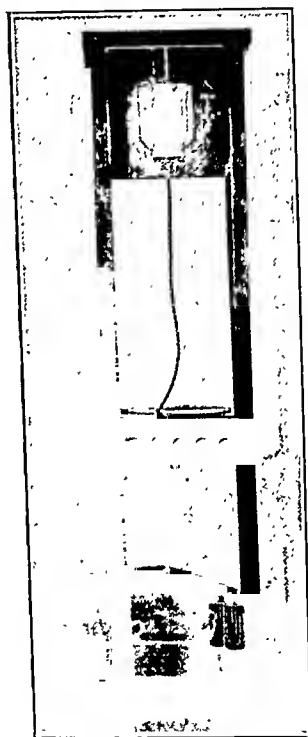
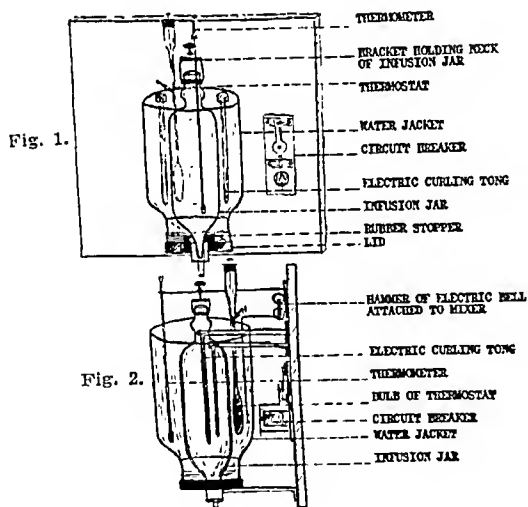


Fig. 3.

The water-bath consisted of a glass jar of one gallon capacity, the bottom of which had been removed. This jar was mounted on the support in an inverted position and held by a strip of brass soldered to the bottom of the lid which had been screwed in place. A brass tube passed through the center of the lid and, surrounding it, a specially shaped rubber stopper was fitted. This in turn received the conical end of the infusion jar which was clamped down on the stopper by a screw clamp which fitted over the neck of the jar above (Figs. 1 and 2). There was provided thus a water-tight compartment, the water-bath, which surrounded the infusion jar. A thermometer and a mechanical mixer which kept the water in constant motion were suspended in the water. A new principle was employed in this mixer in that the sharp edge of the blade, which consisted of a piece of tin, cut the water by a to-and-fro

the content of the outer bag before and after the test period is a measure of the oxygen consumed from the can and is determined by weighing the bag on an ordinary prescription balance. An interpretation of the observed weight difference in terms of volume is made and a suitable correction factor for humidity of the room air calculated. The accuracy is approximately that of current portable closed circuit volumetric apparatus.

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## AN APPARATUS FOR THE THERMAL CONTROL OF SOLUTIONS FOR INTRAVENOUS ADMINISTRATION\*

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J. B. C. ROBINSON, AND A. S. BARBER, LONDON, CANADA

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**D**URING the course of a recent investigation concerning certain practical aspects of intravenous therapy as employed in routine hospital practice, an apparatus was devised for the purpose of maintaining the temperature of an infusion at a constant level. This device has proved sufficiently useful to warrant the presentation of a description of it.

The idea of attempting to regulate the temperature of fluids entering the veins of patients is by no means new. For example, Penfield and Teplitzky<sup>1</sup> recommended that the rubber tube leading from the infusion jar to the needle be suspended in a water-bath heated by means of a copper bar, one end of which protruded from the side of the bath over the flame of an alcohol lamp. Hendon<sup>2</sup> used a thermos bottle as a container for the fluid. Watson<sup>3</sup> led the connecting tube through a water-bath heated by means of an electric light bulb immersed in the water, the current being regulated by means of an electrically controlled thermostat so adjusted as to maintain the desired temperature. Titus and Dodds<sup>4</sup> devised an electrical heating unit which surrounded a glass tube through which the liquid flowed to the needle. Mulholland<sup>5</sup> placed hot water bottles around the container and the delivery tube. Perlstein<sup>6</sup> interposed between the rubber connecting tube and the needle, a glass tube which was strapped to an electric curling tong. Morrison<sup>7</sup> merely suspended the container of the fluid in a specially constructed wooden box in which were mounted several electric light bulbs.

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\*From the Clinical Laboratory, Victoria Hospital.  
Received for publication, June 25, 1934.

which result in a marked brittleness of the tissue. The tissue is fixed for forty-eight hours and washed in running water for twenty-four hours.

*Staining.*—Paraffin sections from 6 to 8 micra thick are brought down to water. The sections are stained as follows:

1. Immerse in 0.25 per cent aqueous aniline blue from sixty to ninety seconds. The basophile cells are stained a deep blue, while the rest of the tissue is stained a very faint blue.

2. Wash in tap water one-half minute. Excessive washing will remove the dye from the basophiles.

3. Immerse in Mayer's hematoxylin ten minutes.

4. Wash in tap water from two to three minutes.

5. Immerse in van Gieson's mixture from sixty to ninety seconds. The mixture is made up by adding 5 c.c. of 2 per cent aqueous acid fuchsine to 100 c.c. of saturated aqueous picric acid. Excessive treatment with van Gieson's mixture will result in a greenish blue coloration of the basophiles.

6. Wash in tap water one minute. Prolonged washing will wash out the van Gieson stain.

7. Transfer sections to 95 per cent alcohol one minute.

8. Dehydrate in absolute alcohol from two to three minutes.

9. Clear in xylol.

By this method the various cellular elements of the human hypophysis are sharply delineated and clearly defined from one another. Nuclear chromatin is stained a purplish brown. The beta (basophilic) granules are stained a dark blue and appear in sharp contrast to the alpha (acidophilic) granules which stain an olive green. The cytoplasm of the chromophobe cells has a light grayish blue color. Red blood cells stain bright yellow, which adds considerably to the ease of studying the sections, especially in glands that show congestion of the blood vessels. The colloid in the anterior lobe stains a variety of colors, varying from yellowish to purple. The colloid in the posterior lobe stains light blue.

Dense collagenous fibers stain deeply with the acid fuchsine, while loose collagenous connective tissue stains lightly either with aniline blue or with acid fuchsine. The capillary walls stain a sharp blue. The stained sections possess a good degree of optical translucency which was not obtained with several of the published stains for the hypophysis.

Stained sections that are now two years old have not shown any significant degree of fading.

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motion and was kept in action by its attachment to the hammer lever of an electric bell. Two electrically heated curling tongs, the handles of which had been removed, were suspended also in the water-bath, one on each side of the intravenous jar. The current which supplied these heating elements was controlled by a mercury-toluol thermostat connected in series with a magnet operating a circuit breaker similar to the method described by Watson.<sup>3</sup>

The thermostat was so adjusted that the temperature of the water-bath remained at 41° C. This temperature was such as to maintain a temperature of 37.5° C. in the fluid as it flowed through the needle into the vein approximately five feet below the jar.

The authors wish to express their appreciation to Dr. E. M. Watson for his valuable suggestions.

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## A SIMPLE DIFFERENTIAL STAIN FOR THE HUMAN HYPOPHYSIS\*

CHARLES SPARK, M.D., NEW YORK, N. Y.

THE increased interest in the physiology of the hypophysis that has been evidenced during the past decade has resulted in the publication of a large number of differential stains for the demonstration of the various cellular elements in the anterior lobe.<sup>1, 2, 3, 4, 5, 6</sup> Romeis<sup>7</sup> lists eleven methods of staining the anterior lobe of the hypophysis. However, the majority of the published methods are complicated and time consuming, and the results obtained are not constant even for pituitaries of the same species of animal.

For the past two years the author has obtained consistently satisfactory results with a simple stain, the individual ingredients of which are available in any pathologic laboratory. The freshly fixed hypophysis of laboratory animals does not stain with the same clearness as that of human glands fixed even twenty-four hours after death.

*Fixation.*—Orth's fluid (Müller's fluid 9 parts, formol 1 part) is superior to fixatives containing mercuric bichloride such as Zenker's or Helly's fluid

\*From Division of Laboratories, Montefiore Hospital.  
Received for publication, May 29, 1934.

number of trypanosomes inoculated and, to a certain extent, on the source and condition of inoculum. Guinea pigs and rabbits when inoculated with nagana blood, derived from a subspecies of *Peromyscus maniculatus*, died likewise by nagana in due time.

# METHOD AND PROCEDURE

1. One or two specimens, of a given subspecies of *Peromyscus maniculatus* (preferably albino *P. m. gambelii*), newly inoculated intraperitoneally are placed in a jar or Cage 1.

2. After the animals in Jar 1 have been infected for about twenty-five days, two drops of their tail blood are taken into a syringe containing 0.5 c.c.

EXPERIMENTAL TR. BRUCEI INFECTION IN PEROMYSCUS MANICULATUS GAMBELII.

(METHOD OF MAINTAINING TR. BRUCEI STOCK IN PEROMYSCUS MANICULATUS.)

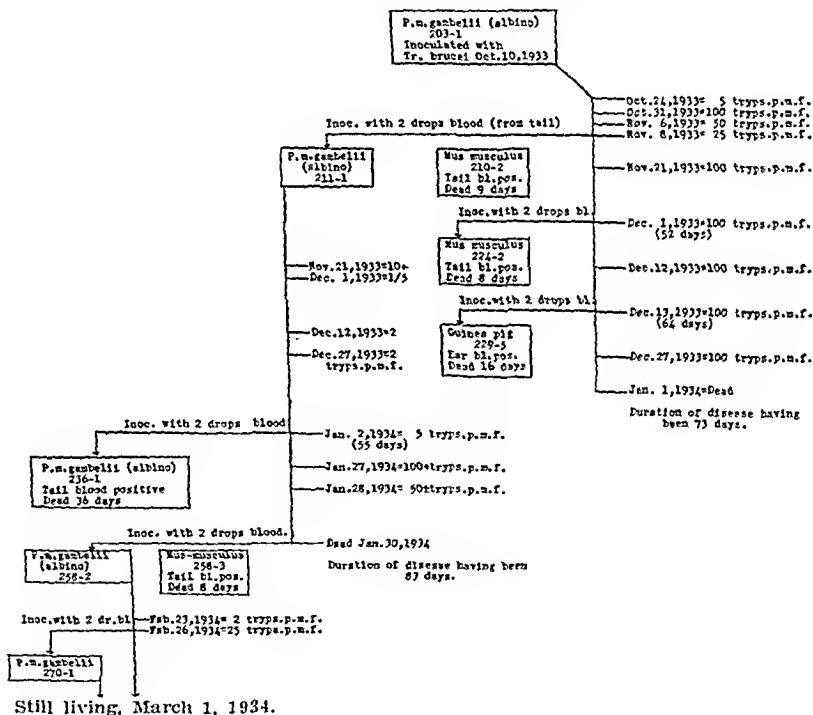


Chart 1.

of physiologic salt solution and inoculated into two other maniculatus which are placed in Jar 2.

3. After the lapse of about fifty days from the last inoculation, two drops of blood obtained from the tail of any previously inoculated animal are inoculated in the mice in Jar 3.

4. Following another fifty days or eighty days from the last inoculation, the mice in the fourth jar are inoculated with the blood obtained from the tail or heart of any of the previously inoculated animals. Usually by this time among the early specimens, one or two may have died. If so, then more specimens are inoculated to replace them.

A METHOD OF MAINTAINING LABORATORY STRAINS OF  
TRYPANOSOMA BRUCEI IN A SUBSPECIES OF  
PEROMYSCUS MANICULATUS\*†

ARDZROONY PACKCHANIAN, PH.D., ANN ARBOR, MICH.

INTRODUCTION

THE laboratory stock of pathogenic trypanosomes such as *Trypanosoma brucei* and *Trypanosoma evansi* usually are kept in *Mus musculus*, *Rattus rattus*, *Rattus norvegicus* or in guinea pigs. The three former species of animals when inoculated with *Tr. brucei* will die within about a week while the latter species will succumb to the disease within three to four weeks. When successive passages of trypanosomes from one animal to another of the same species are made this period is still shorter. The so-called "fixed virus" period of *Tr. brucei* for *Mus musculus* is about three days and for guinea pigs about eighteen days.

In this paper the writer wishes to suggest another species of laboratory animal for maintaining parasitic strains of *Tr. brucei*, *Tr. equiperdum*, and *Tr. evansi*, a method both inexpensive and time saving.

In previous studies,<sup>6, 7</sup> the writer has shown that when various subspecies of *Peromyscus maniculatus* were inoculated intraperitoneally with our strain of *Tr. brucei* the parasites appeared in the circulation of these animals within two or three days. Their number increased gradually until they were very numerous, then they more or less suddenly disappeared from the circulation. This first crisis and the subsequent short latent period were followed with a relapse of trypanosomes. Their number increased again until the blood of the animals was swarming with them. Occasionally an animal died at this stage, but, in the majority of animals the relapse was soon followed by another crisis. The process of frequent crises and relapses followed each other in a more or less regular manner for a number of times, until finally the last relapse of trypanosomes killed the animal. From the first sign of infection until the death of the animals their blood was infective and fatal to susceptible animals, such as *Rattus norvegicus* (laboratory white rats), *Rattus rattus* and *Mus musculus* ("house mice" and so-called "laboratory white mice"†) killing them within five to fifteen days. The time element depended on the

\*From the Hygienic Laboratory, University of Michigan.

Received for publication, May 7, 1934.

†*Maniculatus* represents a species of American deer mice genus *Peromyscus*. Numerous subspecies of *Maniculatus* are widely distributed in their natural habitation on the North American continent.<sup>8</sup>

‡"White mice" in the past has referred to the albino mutant of *Mus musculus*. This term is no longer descriptive, for we have albino mutant of *P. maniculatus gambelii*. The albinism of this strain is typical and complete, genetically it is a simple recessive. This strain can be reared in the laboratory indefinitely.<sup>10</sup> Experimental nagana in albino *P. m. gambelii* and in albino *Mus musculus* is distinctly different.<sup>9, 7</sup>





It is desirable that frequent tail blood examinations and occasionally susceptible animal inoculations be made as long as the *P. m. gambelii* are alive, and that the results be recorded.

#### EXPERIMENTAL DATA

Charts 1 and 2 are representative of experimental nagana in nine subspecies of *maniculatus* thus far studied, i.e., *P. m. artemisiac*, *P. m. bairdii*, *P. m. blandus*, *P. m. gambelii*, *P. m. hollesteri*, *P. m. osgoodii*, *P. m. rubidus*, *P. m. rufinus*, and *P. m. sonorensis*.

Table I represents duration of nagana in *P. m. gambelii* (colored, albino and hairless).

EXPERIMENTAL TR. BRUCEI INFECTION IN PEROMYSCUS MANICULATUS BLANDUS

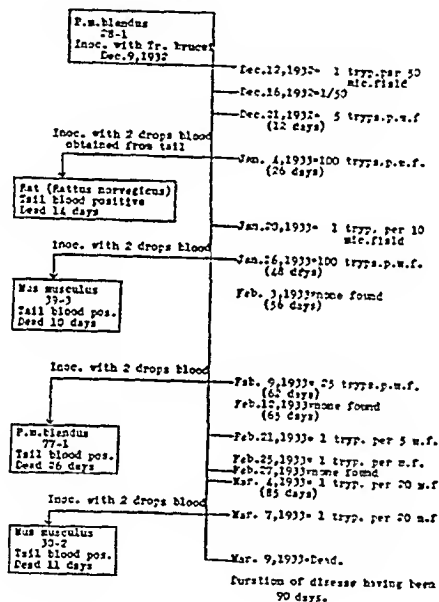


Chart 2.

For the details regarding the relative duration of the disease in various species of *Peromyscus*, the reader is referred to the previous works.<sup>6, 7</sup>

#### DISCUSSION

The method of maintaining *Tr. brucei* in a subspecies of *Peromyscus maniculatus* (American deer mice), is based on several hundred animal inoculations extending over three years. In choosing a species of mice (*Peromyscus*) for the keeping of the laboratory stock, one has to take into consideration the following:

A subspecies of *maniculatus* should be chosen in which *Tr. brucei* can be kept for a fairly long time, but a subspecies in which the trypanosome will eventually terminate the life of the animal. In this regard care must be taken to avoid obtaining a subspecies in which the parasites, after successive passages, will be gradually attenuated. We have at present no evidence that any subspecies of *maniculatus*, once inoculated with *Tr. brucei*, will ever re-

species such as *P. m. rufinus* male with *P. m. gambelii* female, add to the vigor and increase resistance to this particular infection. For this reason it is not advisable to use hybrid offspring for maintaining pathogenic trypanosome stocks, because it is not known how far *Tr. brucei* may be modified during its indefinite sojourn in a given hybrid offspring.

The "ideal" type, therefore, is one in which the length of the *Tr. brucei* infection, after successive passages, remains approximately the same, but which always, of course, ends in the death of the animal not sooner than two or three months. In this "ideal" type the trypanosomes must be detectable in the circulation of the animal in sufficient number at frequent intervals and should not lose their pathogenicity to laboratory animals, such as rats and rabbits, any more than if the stock were kept in guinea pigs.

If the stock of *Tr. brucei* is kept in the following species *P. californicus californicus*, *P. c. insignis*, *P. eremicus anthonyi*, *P. c. eremicus*, *P. c. feticus*, and *P. polionotus polionotus*, it will be just as expensive and time-consuming as if the strain is kept in *Mus musculus*, *Mus bairdii*, or *Rattus norvegicus*. Experimental *Tr. brucei* infection in these species of animal is, as a rule, an acute, progressive and fatal disease, characterized by the constant presence of the parasites in the peripheral blood. Not a single animal of the above species survived longer than fifteen days. If one wishes or is compelled to maintain the stock of *Tr. brucei* in these species, the animal inoculations must be performed every two or three days. On the other hand if the strain is kept in *Peromyscus leucopus noveboracensis* there is greater danger of losing it because the majority of the mice of this species either recover from the disease or alter the virulence of the trypanosomes to such an extent that they will produce the infection in rats, seldom or only after an extremely prolonged incubation period. This delayed test animal infection is particularly noticeable when the subinoculum is taken from the *noveboracensis* a few months after its initial inoculation. After the lapse of a longer period, about one hundred and fifty days, its blood usually is no longer infective to susceptible animals. *Tr. brucei* infection in a small percentage of *P. leucopus noveboracensis* animals is detectable even a year after initial inoculation, or subsequent reinoculations with *Tr. brucei*. For this reason it is considered an extra precaution for preserving the stock to inoculate a few *P. l. noveboracensis* very heavily with *Tr. brucei* once every year. But it must be borne in mind, that not always can one rescue *Tr. brucei* from the individuals of this species a year after its initial inoculation.<sup>6, 7</sup>

#### SUMMARY

1. It is shown that the species of *Peromyscus maniculatus* is a suitable animal for indefinite maintenance of laboratory stocks of pathogenic trypanosomes, such as *Tr. brucei*, and *Tr. evansi*, the minimum period of life being twenty-one days in a few instances. The maximum was two hundred and thirty days, while the general average was about eighty days.

2. *Tr. brucei* during its sojourn in a subspecies of *P. maniculatus* was not attenuated to any extent and the blood of the animals from the first sign of

infection until death was infective and virulent to the susceptible animals, such as *Rattus rattus*, *Rattus norvegicus*, and *Mus musculus*, killing them within five to fifteen days.

*P. m. artemisiae*, *P. m. bairdii*, *P. m. blandus*, and particularly albino *P. m. gambelii* are suggested as convenient and inexpensive animals for maintaining *Tr. brucei* for the purpose of classroom teaching and for research. Other subspecies of *maniculatus* such as *P. m. osgoodii*, *P. m. sonorensis*, *P. m. rubidus*, *P. m. rufinus*, etc., likewise are suitable for maintaining the pathogenic stock of trypanosomes.

I wish to express my sincere thanks and appreciation to Professor F. G. Novy at the University of Michigan for his kind interest in this work. I am also grateful to Dr. Lee R. Dice and Dr. H. W. Feldman at the University of Michigan for supplying me with various species and hybrids of *Peromyscus* used in this investigation.

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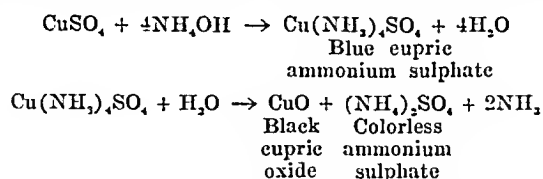
# QUANTITATIVE DETERMINATION OF SUGAR IN THE URINE\*

## AN OFFICE TEST

J. F. KOOGLER, M.D., AND F. STANLEY MOREST, M.D., KANSAS CITY, Mo.

IT IS the purpose of the authors to make available to physicians a quantitative urinary sugar test, which can be performed quickly while the patient is in the office. Response to diabetic dietary or insulin, can be checked and intelligent instructions given. The test was not designed to compete with blood sugar determinations, or lengthy quantitative urinary sugar estimations made in hospital and commercial laboratories. It is not always feasible to subject diabetics to blood sugar examinations on every office visit. This modified Purdy's method with its ease of performance, yet possessing the necessary sensitivity, finds its most extensive use in excluding the presence of pathologic amounts of sugar in routine urinalysis. Patients can determine the percentage of sugar in specimens passed two hours after meals, and bring reports to the physician.

Purdy,<sup>1</sup> in 1889, formulated his test solution and method for the quantitative determination of sugar in the urine. According to Cummer<sup>2</sup> and Webster,<sup>3</sup> Purdy's solution is superior to any copper solution prior to, and including, Fehling's formula. Objections offered to Purdy's solution are instability and ammonia fumes. The solution keeps for months, but constant action of ammonia on cupric sulphate forms cupric ammonium sulphate, which eventually disintegrates liberating cupric oxide as shown in the following chemical equations:



To prevent instability, the original formula was modified by placing copper and ammonia in separate solutions. The two solutions, kept separately until required, remain stable indefinitely. Using 4 c.c. of test-solution, instead of 35 c.c. as needed in Purdy's method, ammonia fumes are not offensive.

### *Composition of Solutions.—*

Alkaline solution	
Potassium hydroxide	23.5 gm.
Glycerin	38.0 c.c.
Strong ammonia (28.33%; U.S.P.; Sp. Gr. 0.9)	350.0 c.c.
Distilled water qs. ad.	500.0 c.c.
Copper solution	
Anhydrous cupric sulphate	4.752 gm.
Distilled water, qs. ad.	500.0 c.c.

\*Received for publication, June 23, 1934.

*Preparation of Solutions.*—Alkaline solution is prepared\* by dissolving 23.5 gm. of potassium hydroxide in 100 c.e. of distilled water, then adding 38 c.e. of glycerin and 350 c.e. of strong ammonia. Distilled water is added to bring volume to 500 c.e.

Copper solution is made by dissolving 4.752 gm. of anhydrous cupric sulphate in 200 c.e. of distilled water, and adding sufficient distilled water to bring volume to 500 c.e.

For convenience in the physician's office, 120 c.e. (4 ounces) of each solution can be made up and placed in glass stoppered bottles. The pharmacist verifies the quantitative accuracy as each new supply of solution is definitely standardized so that 4 c.e. is reduced by 0.00229 gm. of anhydrous dextrose. A "trial-solution" containing 2.2 grains (0.45 per cent) of dextrose to an ounce of distilled water accompanies fresh amounts of test-solutions to the doctor's office. It is preferable that another pharmacist make the "trial-solution." The physician checks the correctness of test-solutions, since 4 c.e. is reduced by 0.5 c.e. of "trial-solution."

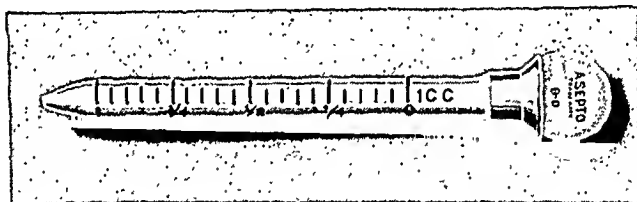


FIG. 1.

*Procedure.*—Into a test tube,\*\* place 2 c.e. each of alkaline and copper solution. A deep blue solution results. Heat the solution to boiling. The 1 c.e. pipette,† shown in the illustration, is filled with urine‡ to be tested. Slowly discharge the urine into the boiling test-solution drop by drop until the blue color begins to fade. Then add urine still more slowly, three to five seconds elapsing after each drop, until the blue color completely disappears, leaving the test-solution perfectly transparent and colorless.§

The authors' pipette is graduated so that each drop is  $\frac{1}{20}$  c.e.

*Calculation.*—The amount of urine required to reduce 4 c.e. of test-solution contains 0.00229 gm. of sugar. Percentage of sugar can be determined with the formula:  $0.229 \div X$ , where X represents number of cubic centimeters of undiluted urine required to effect reduction. Example: 0.5 c.e. of undiluted urine is necessary to reach the end-point.  $0.229 \div 0.5 = 0.45$  per cent. Urines reading 0.2 per cent or under, may be considered negative for glucose.¶ To secure quickly

\*Solutions are obtainable from J. E. Griffin, Apothecary, 216 East 11th Street, Kansas City, Mo., or may be prepared by a graduate pharmacist.

\*\*A test tube previously scored with an ampoule file, indicates the 2 c.e. and 4 c.e. marks. †Asepto Pipette, No. 2025—Special; graduated in 0.05 (1/20) c.e., Beeton, Dickinson & Co., Rutherford, N. J.

‡Selected specimens passed two hours after meals, to which toluene has been added as preservative.

§Upon standing, the test-solution again regains its blue color. This is due to reoxidization and should not be mistaken for imperfect reduction or defect in test-solution.

the results of a test, consult Table I which gives amount of sugar in the urine, both in percentage and in grains per fluidounce.

TABLE I  
RATIOS OF REDUCTION USING 4 C.C. OF TEST-SOLUTION

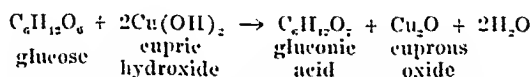
DROPS (1 DROP = 0.05 C.C.)	UNDILUTED URINE		PER CENT OF SUGAR
	C.C.	GRAINS OF SUGAR PER OUNCE	
1	0.05	22.00	4.40*
2	0.10	11.00	2.20
3	0.15	7.33	1.47
4	0.20	5.50	1.10
5	0.25	4.40	0.88
6	0.30	3.67	0.73
7	0.35	3.14	0.63
8	0.40	2.75	0.55
9	0.45	2.45	0.50
10	0.50	2.20	0.45
11	0.55	2.00	0.40
12	0.60	1.83	0.37
13	0.65	1.69	0.34
14	0.70	1.57	0.31
15	0.75	1.46	0.29

\*When the percentage of sugar is above 1.10, dilute the urine with three volumes of water and multiply table figures by 4.

For practical purposes, a medicine dropper, which has been tested\* and found to deliver 20 drops of urine to a cubic centimeter, can be used to obtain accurate results. A 1 or 2 c.c. pipette, graduated in tenths, may be used. In either case, the table of results prepared above is equally applicable, and eliminates solving arithmetical problems.

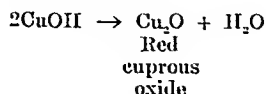
#### DISCUSSION

The reaction† in which glucose is oxidized by cupric ions may be written as follows:



The cupric ion is the oxidizing agent, and is reduced to the cuprous state by glucose in the presence of alkali. Cupric ions in solution are in equilibrium with residual cupric hydroxide, which in turn may be regarded as existing in equilibrium with ammoniacal copper.

Decomposition of cuprous hydroxide to form cuprous oxide follows:



Reduced copper, though very insoluble, is soluble in strong ammonia. This fundamental chemical fact is responsible for the colorless end-point. Reduced copper is not precipitated, but is held in solution by ammonia. The blue color

\*Heat the tip of the medicine dropper and if the drops are too large, draw out the tip of the dropper; if too small, cut off the tip.

†Dr. James C. Rice, instructor in chemistry at Kansas City Junior College, reviewed the chemical interpretations presented.

of the test-solution grows paler as the reduction takes place. When reduction is complete, the blue test-solution becomes suddenly decolorized but no precipitate appears.

Urates may turn the test-solution a light greenish yellow, but this change is always accompanied by a flocculent brown precipitate, or a finely divided white precipitate. In no way can such a change be confused with the transparent and colorless end-point produced by sugar reduction. The test-solution does not react with formaldehyde, albumin, uric acid, morphine, chloroform, acetylsalicylic acid, or sodium salicylate.

#### CONCLUSIONS

These deductions are drawn from the experience gained in performing over 3,000 quantitative sugar determinations with this method.

1. When the end-point is reached, results are read directly from Table I.
2. Small amount of solution and elimination of cumbersome apparatus make the test inexpensive.
3. This method is rapid; the technic is simple; the solutions are stable.
4. Diabetic patients can report percentage of sugar found in postprandial specimens.
5. Advantages of sugar test herein outlined over Benedict's method are: (a) More definite and sharp end-reaction; (b) no loss of time waiting for solution to cool in detecting the presence of 0.3 per cent sugar, or less; (c) short boiling time; (d) no bumping in tube; (e) no false positive reaction with phosphates, urates, morphine, and sodium salicylate; and (f) accuracy of test-solution easily checked.
6. Its widest application is as a test to exclude the presence of abnormal amounts of sugar in routine urinalysis.
7. First quantitative test offered diabetics that is practicable at home.
8. The test is qualitative as well as quantitative.
9. A method for quantitative urinary sugar determination is presented primarily as an office test, to aid physicians to better control diabetic dietary or the use of insulin.

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## A METHOD FOR OVARIAN TRANSPLANTATION ON RABBITS USED FOR THE ASCHHEIM-ZONDEK TEST FOR PREGNANCY\*

KENNETH C. CAMPBELL, MARLBORO, N. J.

IN THOSE laboratories performing a posterior or anterior laparotomy on rabbits which have been injected for the purpose of making a diagnosis of pregnancy, and which are used for the test from three to five times, a new method may be employed to distinct advantage. The mortality rate of the animals may be reduced, and more accurate examination of the ovaries made possible, if the following procedure is used: Repeated entering of the peritoneal cavity for each examination and lifting out of the ovary through the fascial wound is a factor contributing to liability of infection of the animals; then, too, the ovary is likely to become traumatized because of repeated manipulations, the resulting hyperemia tending to interfere with the detection of hemorrhagic follicles.

The advantages of the method of ovarian transplantation here described are: (1) the ovaries are readily accessible; (2) less time is required for the examination as there is only one incision to be made; (3) anesthesia is of short duration and resulting anesthetic deaths are reduced to a minimum; (4) the mortality from infection is greatly diminished; (5) the ovaries are more likely to maintain a normal appearance than is the case if the organs are handled during each examination.

The entire procedure consists of an initial posterior laparotomy, transplantation of the ovaries into the subcutaneous areolar tissue of the back, and subsequent examination by opening the original incision and lifting the skin flaps for inspection of the ovaries.

The technic for the initial operation is described by Goodale and Flanagan on page 58 of the JOURNAL OF LABORATORY AND CLINICAL MEDICINE for October, 1932, as follows:

The rabbit, under ether anesthesia, is placed in the position normally assumed by the rabbit at rest. The lower back is covered with tincture of iodine which is rubbed into the hair with a sponge. The hair may be removed with scissors and it is then found easier to suture the skin. The instruments necessary are a scalpel, mouse-tooth forceps, a hook, scissors, needle and needle-holder, and No. 1 catgut suture. These are placed in a dish of 70 per cent alcohol. The operator, wearing rubber gloves, rinses his finger tips in the alcohol. The operator locates the fifth vertebra about the juncture of the femur with the coxal or pelvic bone. A 4 cm. longitudinal incision is made in the midline and should be bisected by the above-mentioned vertebra. The incision is carried completely through the skin which is then readily separated from the fascia and muscle layer beneath. Because of the looseness of the rabbit's skin the single incision serves for the examination of both ovaries.

\*From the New Jersey State Hospital.  
Received for publication, June 20, 1934.



The skin incision is now pulled to one side until the white line of the lumbodorsal fascia is seen. This is usually about 2 cm. from the midline and vertebral column and is just lateral to the thick sacrospinalis muscle. A 2 cm. incision is made through the lumbodorsal fascia. The intestines are then seen through the parietal peritoneum. The peritoneum is picked up with forceps and is cut, allowing entrance into the abdominal cavity. The hook is then inserted and the ovary brought into view. The ovary is usually found medial to the incision. The hook sometimes engages with the horn of the uterus or the tube, but in either case the ovary is readily found. In a careful operation bleeding is not usually encountered.

From this point on, the procedure differs from the original in that the ovary, instead of being dropped back into the peritoneal cavity is transplanted into the subcutaneous areolar tissue. The ovary is held out through the incision in the fascia and careful suturing is done around it, and the edges of the fascial slit brought together. This suturing is close enough to the tube to prevent prolapse of the ovary into the peritoneal cavity but not close enough to compress the blood supply. After both ovaries have been treated in this manner, the skin is allowed to slip back into normal position and the skin sutured. Subsequent examinations may be made at any desired intervals. When one wishes to observe the ovaries it is necessary only to sever the sutures in the skin and look beneath the flap. When healing around the ovary is well advanced, and the ovary has a normal appearance, the first injection may be given and the effect of the injection upon the ovary observed as frequently as may be desired.

Repeated tests may be done on the same animal, it being necessary, of course, to allow adequate time for the ovaries to return to normal after a positive reaction.

#### EXPERIMENTAL EVIDENCE

Feb. 12, 1933: Ovaries of virgin Rabbit 93 transplanted. Ovaries were observed to be slightly larger than normal average. After the required number of sutures were taken in the fascia and the ovaries found to be secure in their new positions, sterile vaseline gauze was packed between the skin and fascia to prevent adhesions. (In later experiments the gauze was found to be unnecessary and to be a predisposing factor in the few infections encountered.) Sixty hours later an exploratory observation was made and healing was found to be well advanced around the ovaries. No evidences of infection were noted. Gauze was again packed in between fascia and skin.

Feb. 16, 1933: A second exploratory examination was made. Healing was found to be more advanced and the wound clean. Three sutures were taken in the skin. Use of gauze discontinued.

Feb. 20, 1933: The ovaries were again examined. Granulation tissue was found in the fascial wound but not on ovaries which were of normal appearance. Rabbit was injected intravenously with 10 c.c. of urine from woman in labor.

Feb. 20, 1933: Examination revealed the ovaries to be markedly enlarged and congested. Discrete hemorrhagic follicles were noted.

Ether anesthesia was used exclusively on this animal.

Feb. 25, 1933: Rabbit 14. Transplantation was done on left ovary. Nothing was done with right ovary. The results in this animal on subsequent examination, were essentially the same as those of Rabbit 93, except that healing was not so rapid.

March 1, 1933: Rabbit 14 injected with known positive specimen.

March 3, 1933: Rabbit 14. A characteristic positive reaction was grossly demonstrable in the left ovary. An incision was made in fascia on the right side, and the right ovary also showed a positive reaction. Ether anesthesia was used.

March 4, 1933: Rabbit 20. Both ovaries were transplanted, and vaseline gauze was inserted in wounds.

March 5, 1933: Rabbit 20. Gauze removed and ovaries examined. They presented an engorged discolored appearance. A small amount of pus was observed in the fascial wound. On direct smear and gram stain of this pus, many gram-positive cocci occurring in clusters were seen. On plain agar slants these organisms showed the cultural characteristics of *Staphylococcus aureus*.

March 5, 1933: Rabbit 20 was emaciated and extremely weak. An offensive odor was noted as the gauze was removed from the wound. A marked suppurative process was observed in and around fascial wounds. Ovaries markedly swollen and of a dark red color.

March 6, 1933: Rabbit 20 found dead in cage. Death apparently was due to peritonitis. Sodium amytal was used as the anesthetic in this case.

March 8, 1933: First experimental Rabbit 93 and five others, Rabbits 19, 30, 36, 80, and 84 were injected with known positive specimens. Rabbits 19, 30, and 36 had previously had bilateral transplantation and Animals 80 and 84 had had but one ovary transplanted, the left and right, respectively. All were suitable for inoculation in five days. The use of vaseline gauze having been discontinued, no postoperative complications were experienced.

On examination, Animals 93 and 19 were found to be suitable for a second injection fourteen days after the positive reaction. Rabbits 30 and 36 were unsuitable until sixteen and eighteen days had elapsed, respectively. Rabbits 80 and 84 required twenty-one and twenty days, respectively before the ovaries returned to normal. Second injections of all these animals (in Case 93, the third) resulted in normal positives, the second injections being given twenty-five days after the first ones.

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## THE INTRADERMAL TEST IN UNDULANT FEVER\*

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### REACTIONS IN HEALTHY AND INFECTED INDIVIDUALS

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GRANT O. FAVORITE, M.D., AND CURTIS F. CULP, M.D., PHILADELPHIA, PA.

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THE laboratory diagnosis of undulant fever in the past has rested on the agglutination test and blood culture. Of recent years, the intradermal reaction has gained favor as an adjunct to the above procedures. We wish to present in this paper our experience with this reaction.

Fleischner and Meyer,<sup>1</sup> in 1918, found that when the *Brucella abortus* protein was injected into the skin of infected guinea pigs, it caused a definite local reaction. In studying 100 children they obtained a positive reaction in 2. Burnet,<sup>2</sup> in 1922, using a filtrate from killed *Brucella melitensis*, obtained positive reactions in cases of undulant fever. Some investigators (Trenti,<sup>3</sup> Omer and Massot,<sup>4</sup> Dubois and Sollier<sup>5</sup>) using his technique, obtained similar results. On the other hand, insufficient specificity has been claimed by others (Brugi,<sup>7</sup> Sorge,<sup>8</sup> Bua,<sup>9</sup> Tapia and DelValle<sup>10</sup>). Enough evidence has accumulated to show that the use of a bacterial suspension is superior to the filtrate (Fornace and Bua-Fazio,<sup>11</sup> Mitra,<sup>12</sup> Bua,<sup>9</sup> DeFermo,<sup>13</sup> Leavell and Amoss<sup>11</sup>) and is less likely to give a false positive reaction. The reaction with the bacterial suspension has proved specific in known cases of undulant fever and negative in other diseases (Sensenich and Giordano,<sup>15</sup> Giordano,<sup>16</sup> Simpson and Frazier,<sup>17</sup> DeFermo<sup>13</sup>).

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\*Received for publication, June 26, 1934.

Using a fat-free filtrate, Levin<sup>18</sup> and Goldstein<sup>19</sup> obtained comparable results with less marked local reaction. Leavell and Amoss<sup>14</sup> claim more specific reaction with heat killed suspensions than with extracts. A single strain of the *Brucella melitensis* group is sufficient as an antigen in performing the test, since it has been found that other members of the group give similar results (Yeckel and Chapman<sup>20</sup>).

Our object was to establish the reaction of the normal individual to *Brucella* antigen and to compare it with reactions in known cases of undulant fever. In the course of our study, we have observed several interesting findings, which we feel will prove of value in the performance and interpretation of this valuable reaction.

*Antigen Used.*—The antigen was made from a strain of *Brucella* isolated from a human case of undulant fever, of probable bovine origin, and grown artificially on plain agar for one year. The organism was grown for seventy-two hours on plain agar, emulsified with 0.5 per cent phenol in physiologic salt solution, and killed by heat at 56° C. for one hour. It was adjusted to a density of 800 million organisms per c.c. when compared with a barium sulphate standard, and tested for sterility on liver infusion agar for one week at 37° C.

*Nonspecific Reaction.*—Ninety healthy medical students were tested for the presence of agglutinin and one was found to be positive in dilution of 1:250. He was excluded from the list of controls. In order to determine the nonspecific reaction, 0.1 c.c. of the antigen was injected intradermally on the flexor surface of the forearm. Subsequent events showed us that a better site for injection was the deltoid region, since positive reactions may persist for a long time. For the average reaction there appeared at the end of twenty-four hours a papule 0.5 cm. in diameter (the size of the original wheal), surrounded by an area of erythema 2 cm. in diameter. In forty-eight hours the papule and the area of erythema were about one-half the size existing at the end of twenty-four hours. In seventy-two hours the papule disappeared but the erythema persisted, and gradually disappeared by the end of one hundred twenty hours. This reaction seemed rather marked for a negative test. Using the same antigen diluted to 80 million organisms per c.c. and attenuating its potency by keeping it in the refrigerator for six months, it was again used on 40 controls who consisted of surgical patients with negative undulant fever history and absent *Brucella* agglutinins. The reaction was greatly modified. A papule, 0.5 cm. in diameter, surrounded by a small area of erythema, gradually disappeared by the end of forty-eight hours. This was compatible with reactions obtained with other organisms. Twenty-five patients in this group were retested with a mixed vaccine containing *E. coli*, pneumococcus, staphylococcus, and streptococcus. They developed a similar papule at the site of injection surrounded by an area of erythema about 2 cm. in diameter which disappeared at the end of seventy-two hours.

*Specific Reaction in Undulant Fever.*—Ten patients with undulant fever in various stages of the disease were tested with the diluted and attenuated antigen. Five were tested during the first month of the illness; 2, during convalescence; and three, one to two years after recovery. The latter patients still had agglutin-

ins, varying in titer from 1:100 to 1:500. The intradermal reaction in these patients consisted of a papule 0.5 cm. in diameter, surrounded by an area of erythema 1 to 2 cm. in diameter at the end of twenty-four hours. The papule assumed a bluish red color. During the first week, there was a gradual decrease in the size of erythematous area, until it merged with the central papule, which, by the end of ten days, appeared as a firm, indurated nodule, measuring 0.5 to 1 cm. in diameter. During the second week the reaction subsided, and persisted as a red area with some induration. Desquamation of the overlying epithelium took place at this stage. In all except one, the reaction continued for several weeks longer. All had local soreness which lasted for forty-eight hours. In no instance was the reaction of sufficient severity to cause local necrosis. These results compare favorably with those of Yeckel and Chapman.<sup>20</sup>

*Positive Reaction in Controls.*—One of the students in the control group, as previously mentioned, had an agglutination titer of 1:250. He gave a strongly positive skin reaction. His history showed that he had ingested raw milk for many years, and still does when he returns home during vacation. Two students with a positive reaction but with absent agglutinins gave histories of exposure to cattle, ingestion of raw milk, or febrile illness. The original undiluted antigen was used on one of these students with marked local reaction which persisted for several months.

*Scarification Test.*—Since the intradermal reaction causes local manifestations which could be less severe, it was thought that it could be moderated by placing the antigen on a scarified skin surface. The same technic was used as in the performance of the Von Pirquet test for tuberculosis and the scarification method for smallpox. Furthermore, a small pledget of cotton saturated with the antigen was placed over the scarified area and held in place by adhesive tape. A slight border of erythema developed in the positive cases, but was not sufficient for diagnostic purposes.

#### COMMENT

With a properly prepared antigen consisting of the whole bacterial suspension of *Brucella abortus*, a definite intradermal reaction was obtained which caused no serious local reaction and left no doubt as to its positiveness. The severity of the reaction depended somewhat on the amount and potency of the antigen. These factors were altered by dilution and aging of the bacterial emulsion so that when used on controls, the nonspecific reaction disappeared in forty-eight hours. Such an antigen caused a definite prolonged reaction in cases of undulant fever which compared favorably with modified antigens used by others. Even in inexperienced hands, the line of demarcation between a positive and negative test was such that it left no room for doubt, as it often happens in skin reactions. The negative reaction, even with the use of a heavy, potent, bacterial emulsion, usually disappeared at the end of five days, and surely at the end of the first week. The positive reaction continued after this time as a firm, indurated, red nodule which persisted for several weeks. The proper time for reading the test, therefore, would be at the end of the first week with subsequent observations.

During the course of our study several interesting observations were made which are worth recording. In two positive cases, a subcutaneous injection of

the vaccine for therapeutic purposes caused a reactivation of the local reaction after it had subsided. Because of the inaccessibility of the other patients with a positive reaction, it was not tried on the others. The phenomenon was not observed in the controls. One of the students with agglutinins in the blood, and a positive intradermal reaction, returned home for the Christmas holidays. His trip home took place during the second week of the reaction. While home he drank large quantities of raw milk. A reactivation of the nodule, with widening of the erythematous zone occurred. No doubt, the milk which was probably infected, had a direct bearing on the reactivation of the lesion.

To show the specificity of the test we cite the following case: A colored male patient suffering with an obscure febrile illness gave a positive Widal test in a dilution of 1:50 and a positive agglutination test for *Brucella abortus* in a dilution of 1:100. The intradermal reaction was negative. At autopsy the patient was found to have miliary tuberculosis.

Thirty-five students in the control group were tested for agglutinins five months after their single intradermal injection of the antigen. No agglutinins were found at the end of this time.

#### CONCLUSIONS

The use of a bacterial suspension of killed *Brucella abortus* gives a specific intradermal reaction in undulant fever which is easily distinguished from a non-specific response.

The bacterial suspension may be adjusted by dilution and attenuation to the desired reaction, as determined by preliminary tests on normal human individuals.

The use of the scarification technic in performing the test does not give conclusive results.

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The amounts discharged in liters per minute are then plotted against the amount received by the spirometer in liters per minute, as shown in Figs. 1

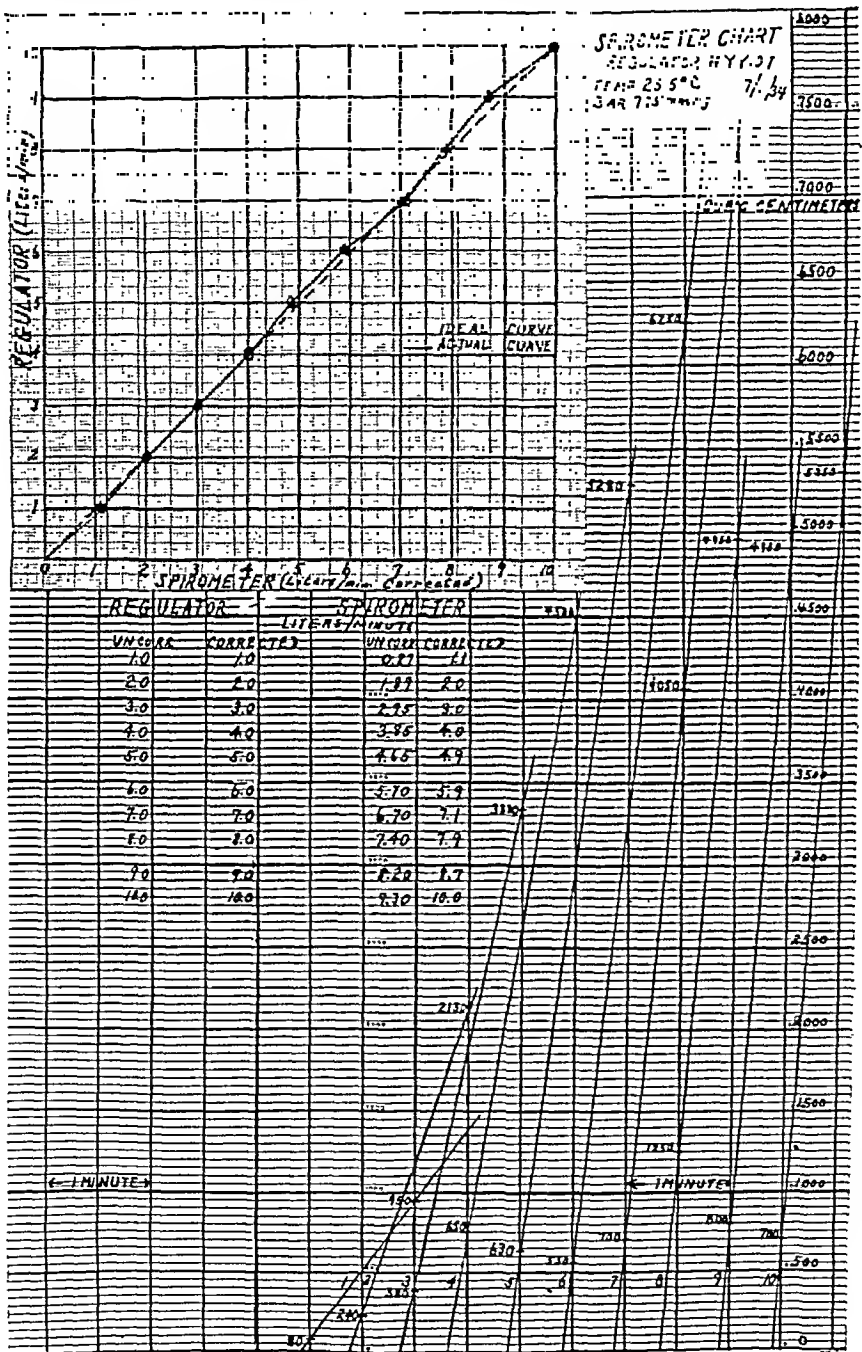


Chart 2.—Spirometer chart. Regulator Y-107, float gauge.

and 2 (inset). Corrections are first made for temperature and barometric pressure and for error in the spirometer scale.

At Harlem Hospital, regulators are calibrated every two weeks, and if there is reason to suspect inaccuracy, they are calibrated more frequently.

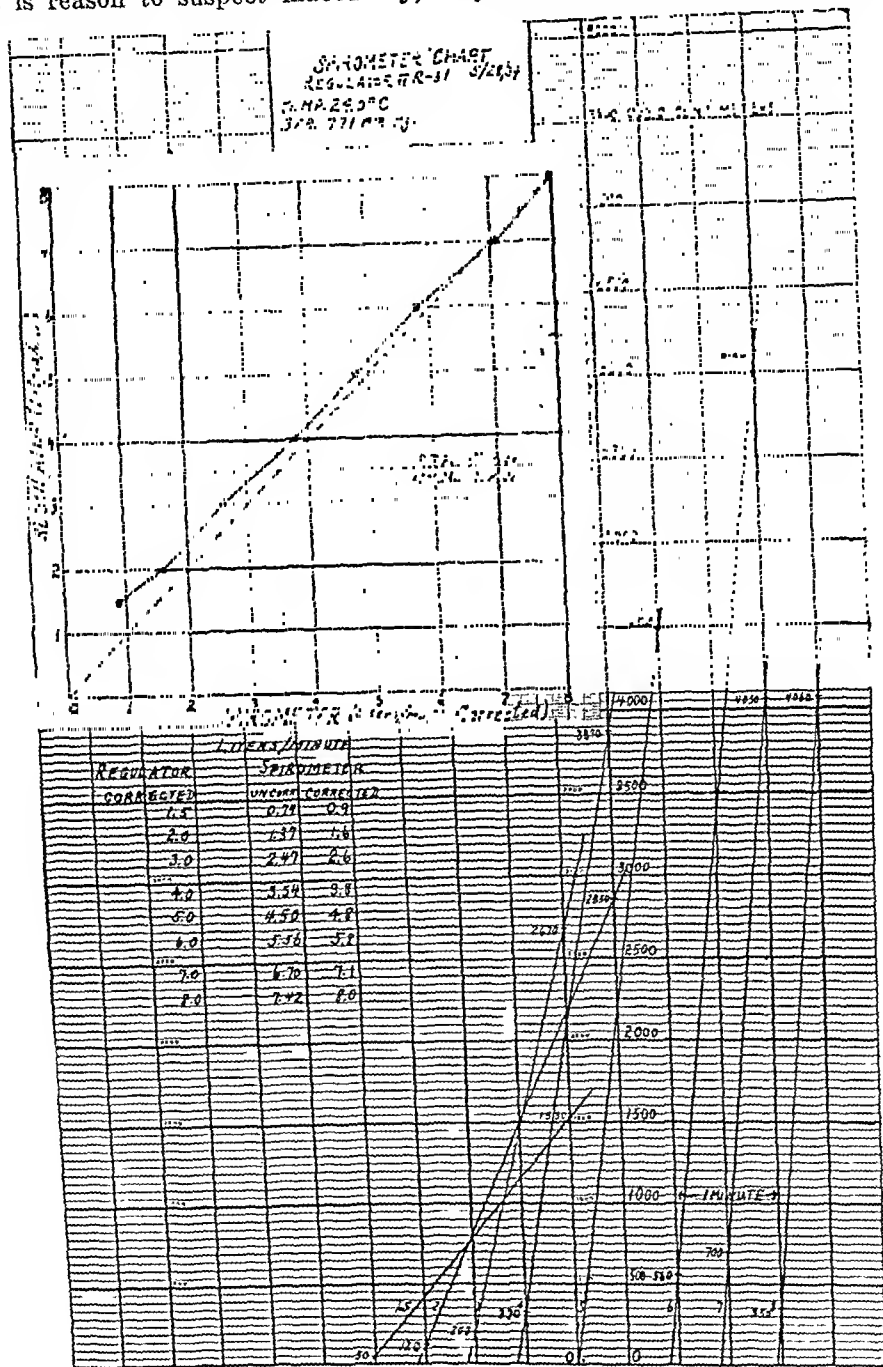


Chart 3.—Sphygmometer chart. Regulator No. R-31. Bourdon tube; dial gauge.

They are run at the various rates with a float or flow gauge in series. The float gauge was previously calibrated by the method described. From time

to time a permanent record is made directly from the regulator by discharging oxygen into the gasometer.

If great accuracy is required, the spirometer or gasometer of the basal metabolism apparatus should have its scale checked. For this purpose a flow gauge may be used after it has been calibrated by weighing the oxygen discharged at given rates (liters per minute) over a measured interval at a known temperature and barometric pressure. After correction for temperature and barometric pressure, the values obtained by discharging oxygen through the gauge into the spirometer are plotted against the values for the gauge as obtained by weighing the oxygen discharged. This curve becomes the standard of reference for the values obtained for other flow gauges.

For determining the weight of oxygen discharged there is required a beam scale sensitive to  $\pm 5$  gm. (silk scales) when the weight on the platform is 70 Kg. The full tank of oxygen (closed) with regulator attached, is placed on the platform. The tank is open and the regulator tested for leaks with a soapsuds film. If there are no leaks, the cylinder is accurately balanced, using weights on the platform. About 1 Kg. of oxygen is then discharged at a known rate (10 liters per minute will require about seventy-five minutes). The flow is then stopped and an accurate weighing is obtained by placing on the platform the required weights of a chemical balance. The weight added will be the weight of the oxygen discharged. The spirometer scale is then corrected for temperature, barometric pressure and the errors, if any.

The formula for calculating the true flow in liters per minute at 70° F. and 29.92 inches Hg follows:

$$Q_o = \frac{W T_o}{t D T_s}$$

$Q_o$  = true flow in liters per minute reduced to conditions—normal atmosphere (29.92 in. Hg) and 70° F. (294.2° Absolute).

$W$  = weight of oxygen in grams (observed).

$t$  = time in minutes (observed).

$D$  = density of oxygen in grams per liter at "normal temperature and pressure." 1.429.

$T_o$  = normal temperature (0° C. = 273.1° Absolute). (Observed.)

$T_s$  = standard reference or base temperature (70° F. = 294.2° Absolute).

$$Q_o = \frac{W}{t} \times \frac{294.2}{1.429 \times 273.1} = 0.7539 \frac{W}{t}$$

The lower the flow, the longer the discharge must last so that at 5 liters per minute,  $t = 120$  minutes, and at 1 liter per minute,  $t = 600$  minutes.

Example: We set flow meter at 10 liters per minute, discharged gas for 61 minutes. Weight of oxygen, 805 gm.

$$Q_o = 0.7539 \frac{805}{61} = 9.95 \text{ l/min. (by weight)} = 10.0 \text{ l/min. (indicated on gauge).}$$



## SUMMARY

There are presented:

1. A rapid, simple method for the calibration of clinical oxygen regulators, which depends upon the use of a clinical basal metabolism gasometer or spirometer.

2. The method for indirectly calibrating the spirometer by weighing the discharge of oxygen through a clinical oxygen regulator equipped with a variable orifice flow meter.

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THE COSTA REACTION\*

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RESULTS IN TWO HUNDRED CASES

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R. H. KAMPMEIER, M.D., F.A.C.P., NEW ORLEANS, LA.

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SEVERAL years ago, I became interested in the use of the Costa reaction for prognosis in the clinical course of pulmonary tuberculosis. Concurrently with the use of the test in pulmonary tuberculosis, I have thought it wise to use it in a variety of diseases so that the effect of fever, syphilis, and neoplasm may be noted. The advantage the Costa reaction has over the sedimentation test is that it can be performed in a few minutes with an amount of blood so small it does not necessitate venipuncture. This latter fact is of especial value in children and in some adults.

## TECHNIC OF TEST

The technic of the Costa reaction is about the same for all the various authors. Slight variations are recorded especially with regard to the degree of reaction as based on the rapidity of its occurrence. The test as done by the author is as follows.

In a small serology tube are placed 1.5 c.c. of a 2 per cent procaine solution made with normal saline. To this is added 0.1 c.c. or 3 drops of a 5 per cent sodium citrate solution. (If blood was used from that collected in a citrated bottle for sedimentation time, this step was omitted). Next is added either 3 drops of blood from a skin puncture, or 0.1 c.c. obtained by venipuncture; the author uses most frequently a tuberculin syringe with hypodermic needle. The tube is then centrifuged from five to eight minutes. As a final step 1 drop of formalin is added, dropped into the solution and not allowed to run down the side of the tube.

A positive reaction is the development of a white cloud of varying amount, and with variable speed, just above the layer of red blood cells. It appears

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\*From the Departments of Medicine, Louisiana State University Medical Center and Charity Hospital of Louisiana, New Orleans, La.  
Received for publication, July 13, 1931.

as a bluish-white haze suggesting tobacco smoke, readily seen against a black background. All authors agree that fifteen minutes is the limit for the reaction, since some normal persons apparently give a reaction after this time. The author has designated the degree of reaction on the plan as suggested by several authors, though here the ideas differ as to the time limits to be set for the various degrees. In this study, a "strongly positive" indicates that the cloud has appeared within three minutes after the addition of formalin. If the cloud appears between three and ten minutes, the designation of "positive" is used. A reaction appearing between ten and fifteen minutes is considered "questionable." No reaction at the end of fifteen minutes is declared "negative."

Some writers say that instead of centrifugation, the procaine-citrate-blood mixture may be allowed to stand twelve hours, and then the test may be completed. In this series the mixture was centrifuged.

#### LITERATURE

In 1923, Costa,<sup>1</sup> while searching for a biologic test for pregnancy found that the blood of pregnant women reacted with procaine and formalin in a manner different from the blood of nonpregnant normal women. He also found such reaction occurred in the presence of active tuberculosis and in tertiary syphilis of the nervous system.

Since Costa first described the reaction, there have been numerous references to it in the European literature. Rio,<sup>2</sup> in trying to establish the importance of procaine, used stavain, cocaine and orthoform, but found the sediment so labile with these that the test was impracticable. In the physicochemical studies of Trojan and Pongor,<sup>3</sup> the hydrogen ion concentration of the reaction solutions was found to be of no significance. Their biologic experiments revealed that no reaction occurs with blood serum, that positive results are obtained only with blood plasma of the actively sick. They believe the reaction is tied up with plasma fibrinogen, for defibrinated blood or plasma will not give a response. However, extracted fibrinogen will not give the test but this may probably be explained on the complicated steps of such extraction. The precipitate obtained in the test may be washed with distilled water and centrifuged, and still a positive test will be obtained up to and including the fourth washing, indicating that the absorbed procaine can be washed out. All authors agree that the physics or chemistry of the Costa reaction is not understood. Costa himself has never stated why he chose procaine for a test for pregnancy.

The clinical literature deals mainly with the use of the test in tuberculosis. Ziegler<sup>4</sup> accredits Vercelli with being the first to use it extensively in this disease. Ladeek,<sup>5</sup> in using the test in 200 women and girls, found the reaction negative in the nontuberculous and in the inactive tuberculous cases. In those with exudative processes the rate was rapid, and slower but positive in the fibroid type. He felt the rapidity and degree of the test was quite parallel to the sedimentation test, but generally less sensitive. However, the Costa reaction was not affected by menstruation as was the sedimentation test. Nüssel and Helbach<sup>6</sup> after performing 698 tests in 213 children found the reaction bore the same relation to disease as the sedimentation test, though less sensitive.

In 352 cases thought to be active pulmonary tuberculosis on a physical basis, Trojan and Pongor<sup>3</sup> found 90.6 per cent reacting positively. Of 282 cases with cavity, 91.8 per cent were positive. Among the active cases, 199 or 56 per cent had positive sputum, 189 of these gave a positive Costa reaction. In 448 inactive cases, the Costa reaction was negative or weakly positive in 88.7 per cent; among the 50 positive reactors were several pregnant women and some with diseases of other organs. Penn,<sup>7</sup> in a study of 130 tuberculous cases, found the test to be an adjunct in determination of activity.

Lowins<sup>8</sup> found the Costa reaction checked closely with the sedimentation test. Ziegler<sup>4</sup> felt that though the test is less sensitive than the sedimentation test, it was just as good for

practical use. Altschuler<sup>8</sup> believed the reaction to be of prognostic value in bone tuberculosis, and that it showed positive changes before clinical complications made themselves manifest. Ziegler<sup>4</sup> quotes Schwartz and Rubenstein as concurring in this belief and as feeling that the reaction is more sensitive than the sedimentation test. They reported the test as being strongly positive in syphilis, gonorrhea and in the presence of tumors. Cullmann,<sup>2</sup> after using the Costa reaction in cases of tuberculosis and carcinoma, found it to be more sensitive than the sedimentation test. Rubenstein,<sup>10</sup> in a study of seventy-five cases of laryngeal tuberculosis, concluded that the Costa test is of great value in differentiating simple from tuberculous laryngitis, and that it is an aid in the determination of activity as a guide to treatment.

### RESULTS

The Costa reaction was used in 200 cases, covering a wide variety of pathologic conditions. These cases were all chosen from the author's medical ward and tuberculosis wards, and some from the tumor clinic, of Charity Hospital. The tests were all performed by the author. Choice of cases was made with the objective of having fair-sized groups of cases of pulmonary tuberculosis, tumors, and a variety of miscellaneous conditions with and without infection. The cardiac group is rather large for this type of study, but may be included legitimately because of the large number of syphilitics, since some European authors hold that syphilis gives a positive Costa test.

In the tuberculosis group, there are 49 cases (Table I). The diagnosis of tuberculosis was based on both positive sputum and x-ray in 35, positive sputum alone in 2, and on the x-ray in 7. The other 5 cases had pleurisy with effusion considered to be tuberculous. Sixteen or 32.6 per cent gave a strongly positive and 18 or 36.7 per cent a positive reaction; 5 or 10.2 per cent reacted questionably and 10 or 20.4 per cent were negative. Of the whole group, 7 were afebrile while under observation and these supplied 4 of the negative or questionably reacting cases.

TABLE I  
COSTA REACTION IN TUBERCULOSIS

DIAGNOSIS	NUMBER	STRONGLY POSITIVE	POSITIVE	QUESTIONABLE	NEGATIVE
Positive sputum and x-ray	35	12	15	3	5
Positive sputum (no x-ray)	2	-	-	1	1
Positive x-ray (neg. sputum)	7	2	2	1	2
Pleurisy with effusion (no sputum or x-ray)	5	2	1	-	2
Total	49	16	18	5	10
Percentage		32.6	36.7	10.2	20.4

Thirty-three patients comprise the tumor group (Table II). Diagnosis was based on biopsy or autopsy in 22, on the x-ray in 10, and on bronchoscopy in 1. Of the total, 5 or 15.1 per cent gave a strongly positive reaction; 19 or 57.5 per cent were positive; 2 or 6 per cent reacted questionably; and 7 or 21.2 per cent were negative. However, if the 21 proved cases of malignancy are considered, the following is found: 19 or 90.4 per cent showed either a strongly positive or positive reaction, and 1 each or 4.7 per cent, a questionable and a negative test.

TABLE II  
COSTA REACTION TO TUMORS

DIAGNOSIS	BASIS FOR DIAGNOSIS	NUMBER	STRONGLY POSITIVE	POSITIVE	QUESTIONABLE	NEGATIVE
Carcinoma floor mouth	Biopsy	1	-	1	-	-
Carcinoma tongue	Biopsy	1	-	1	-	-
Carcinoma hard palate	Biopsy	2	-	2	-	-
Carcinoma maxillary sinus	Biopsy	1	-	1	-	-
Carcinoma larynx	Biopsy	1	1	-	-	-
Carcinoma bronchus	Biopsy	1	1	-	-	-
	Autopsy	1	-	1	-	-
	X-ray	4	1	1	-	2
Pulmonary metastases (primary unknown)	X-ray	1	-	1	-	-
Carcinoma esophagus	Biopsy	1	-	1	-	-
Carcinoma stomach	X-ray	3	-	-	1	2
	Biopsy	4	-	4	-	-
Carcinoma rectum	Biopsy	1	-	1	-	-
Carcinoma pancreas	Autopsy	1	-	1	-	-
Carcinoma skin	Biopsy	1	-	1	-	-
Fibroadenoma of breast	Biopsy	1	-	-	-	1
Carcinoma cervix (treated)	Biopsy	1	-	-	1	-
Carcinoma penis	Biopsy	2	-	2	-	-
Hypernephroma	X-ray	1	1	-	-	-
Hypernephroma (rib metastasis)	Biopsy	1	1	-	-	-
Spindle-cell sarcoma pharynx	Biopsy	1	-	-	-	1
Mediastinal tumor	X-ray	1	-	1	-	-
Polypus, bronchus	Bronchoscopy	1	-	-	-	1
Total		33	5	19	2	7
Percentage			15.1	57.5	6	21.2

TABLE III  
COSTA REACTION IN DISEASE OF HEART AND AORTA

DIAGNOSIS	NUMBER	STRONGLY POSITIVE	POSITIVE	QUESTIONABLE	NEGATIVE
Aortic insufficiency	12	1	1	-	10
Aortitis	11	-	-	-	11
Arteriosclerotic heart disease	12	2	1	1	8
Coronary closure	1	-	1	-	-
Hypertensive heart disease	6	1	1	-	4
Rheumatic heart disease	2	-	2	-	-
Total	44	4	6	1	33
Percentage		9.0	13.6	2.2	75
Congestive failure	34	4	4	1	25
No congestion	10	-	2	-	8

In Table III is found an analysis of the cardiac group, consisting of 44 cases. Of these 33 or 75 per cent were Costa negative. In the syphilitic group, cases of aortic insufficiency and aortitis, 23 in number, were found 21 or 91.3 per cent who were Costa negative. Twenty-five of the total cardiac group had a positive blood Wassermann test and of these, 20 or 80 per cent gave a negative Costa reaction.

In addition to the 23 cases of cardiovascular syphilis, the test was carried out in 10 cases showing other active syphilitic lesions (Table IV). Of this group, only 2 or 20 per cent showed a positive, 1 gave a questionable,

TABLE IV  
COSTA REACTION IN SYPHILIS

DIAGNOSIS	NUMBER	STRONGLY POSITIVE	POSITIVE	QUESTION-ABLE	NEGATIVE
Lymphadenitis	1	—	—	—	1
Cutaneous (secondary)	1	—	—	—	1
Nephrosis	1	—	—	—	1
Hepatitis	1	—	—	—	1
Osteitis (ribs)	1	—	1	—	—
Paresis	2	—	—	—	2
Meningovascular	3	—	1	1	1
Total	10	—	2	1	7
Percentage			20	10	70
Positive Wassermann in whole series	46	5	7	3	31
Positive Wassermann in cardiac group	25	2	2	1	20

and 7 or 70 per cent showed a negative Costa reaction. The blood Wassermann test was done in all the 200 cases included in this study. Forty-six of these had a positive blood Wassermann reaction, and of these 31 or 67.3 per cent gave a negative Costa reaction, and 3 or 6.5 per cent a questionable one. Among the 12 positive cases are included such conditions as pulmonary tuberculosis, lung abscess and the like, which better explain the positive result.

The results in a group of 37 cases, presenting a miscellaneous group of conditions with infection of one type or another, are listed in Table V. We see that 4 or 10.8 per cent were strongly positive, 14 or 37.8 per cent were positive, 6 or 16.2 per cent were questionable and 13 or 34.8 per cent were negative. Twenty-three of the whole group presented fever at the time the test was done. In the febrile group, 13 or 56.5 per cent showed either a strongly positive or positive Costa test, while 12 or 43.5 per cent were either questionable or negative.

Twenty-seven cases of miscellaneous diseases are grouped together in Table VI. Of these, only 1, a case of sickle-cell anemia, had fever. Two or 7.4 per cent were strongly Costa positive, 4 or 14.8 per cent were positive, 1 or 3.7 per cent was questionable, and 20 or 74 per cent were Costa negative.

#### DISCUSSION

The Costa reaction is being used by the author on his tuberculous wards in conjunction with the sedimentation test and differential blood cell picture from a prognostic standpoint. When a group of tuberculous patients has been studied for a long enough period of time, a comparison will be made between this and other prognostic criteria. As a preliminary study, it was thought advisable to use the test in a variety of pathologic conditions in order to evaluate the factors influencing the reaction. Therefore, this communication deals with the reaction in 200 selected cases.

In active pulmonary tuberculosis, the Costa reaction is found to be positive in a high percentage of cases, 70 per cent having been either strongly positive or positive with another 10 per cent being questionable. In this paper,

I will not go into the matter of activity or type of pulmonary lesion as related to the degree of the Costa reaction. Suffice it to say that among the ten negative cases were some with minimal lesions well under control and some treated by artificial pneumothorax which was followed by cessation of sputum and fever.

TABLE V  
COSTA REACTION IN MISCELLANEOUS INFECTIONS

DIAGNOSIS	NUMBER	FEVER	STRONGLY POSITIVE	POSITIVE	QUESTION-ABLE	NEGATIVE
Bronchiectasis	2	1	-	-	-	2
Lung abscess	6	6	2	4	-	-
Pneumonia	4	4	-	3	1	-
Pneumonia (nonresolution)	1	-	1	-	-	-
Pneumonia (convalescence)	2	-	-	1	-	1
Typhoid fever	3	3	-	2	1	-
Peritonitis (tuberculous)	3	3	-	1	-	2
Osteomyelitis (tuberculous)	1	-	1	-	-	-
Adenitis (tuberculous)	1	-	-	-	-	1
Malaria	4	1	-	1	1	2
Arthritis, acute	1	1	-	-	1	-
Lymphadenitis, suppurative	1	1	-	1	-	-
Tonsil, necrotic ulcer	1	-	-	-	1	-
Rheumatic fever	1	1	-	-	-	1
Pyelitis	1	1	-	-	-	1
Subdiaphragmatic abscess	1	1	-	-	-	1
Amebic dysentery	3	-	-	1	1	1
Catarrhal jaundice	1	-	-	-	-	1
Total	37	23	4	14	6	13
Percentage			10.8	37.8	16.2	34.8

TABLE VI  
COSTA REACTION IN MISCELLANEOUS CONDITIONS

DIAGNOSIS	NUMBER	STRONGLY POSITIVE	POSITIVE	QUESTION-ABLE	NEGATIVE
Pellagra	3	-	-	-	3
Peptic ulcer	4	-	-	-	4
Hypothyroidism	1	-	-	-	1
Hookworm infestation	1	-	-	-	1
Nephritis, subacute	1	-	1	-	-
Nephritis, chronic glomerulo	3	1	1	-	1
"Nephrosis," juvenile	1	-	-	-	1
Leucemia, myelogenous	1	-	-	-	1
Portal cirrhosis	1	-	1	-	-
Hypertension, malignant	1	-	-	-	1
Sickle-cell anemia	1	-	1	-	-
Hepatomegaly (unknown cause)	1	1	-	-	-
Diarrhea, chronic (cause undetermined)	1	-	-	1	-
Neurocirculatory asthenia	1	-	-	-	1
Asthma, bronchial	1	-	-	-	1
Mesenteric cysts (multiple)	1	-	-	-	1
Diabetes mellitus	1	-	-	-	1
Epilepsy	1	-	-	-	1
Schizophrenia	1	-	-	-	1
Progressive muscular atrophy	1	-	-	-	1
Total	27	2	4	1	20
Percentage		7.4	14.8	3.7	74

Occasional mention is made in the European literature of the Costa reaction in the presence of newgrowth, but not much data is given. A strongly positive or positive reaction was obtained in 72.6 per cent of 33 such cases in this study. More significant, however, are the findings in 21 cases of malignancy proved microscopically. Of these, 90.4 per cent were Costa positive. This fact is surely of interest, though no conclusions could possibly be drawn from only 21 proved cases. It is planned to study the reaction before and after treatment by operation and radiotherapy to find whether the test is of any prognostic value in the clinical course of malignancy.

As was to be expected, no significant findings were revealed in the study of the cardiac group. Positive reactions in cardiovascular disease are difficult to explain except to note that the presence of advanced anasarca, marked hepatic congestion, pulmonary congestion, and nephritic involvement with nitrogen retention may have been factors influencing the test. Certainly it seems that the Costa test will offer nothing in the study of cardiac disease.

The findings in this study do not agree with those of certain European writers with respect to the Costa reaction in syphilis. In 23 cases of cardiovascular syphilis, the test was negative in 21. The reaction was generally negative in 10 other patients suffering from active syphilitic lesions. Some positive reactions occurred in patients with positive blood Wassermann reactions, but such usually also suffered from pulmonary tuberculosis, lung abscess, and the like. It seems certain that the presence of syphilis does not influence the Costa reaction.

Fever in itself probably does not cause a positive reaction. Of 37 cases in which infection played a part, 23 were febrile. Only about one-half of these gave a positive reaction. There are not enough cases of any one type of infection from which to draw conclusions as to the type of Costa reaction to expect. Lung abscess, of which there are 6, and pneumonia, of which there are 4, reacted in each case with a positive result. However, it does not appear probable that this reaction will be of value in diagnosis or prognosis in various infections. We do learn from this group, however, that fever in itself does not account for a positive reaction.

In the miscellaneous conditions nothing of interest was found. Positive reactions occurred in nephritis with nitrogen retention, but this fact is not of importance.

#### CONCLUSIONS

1. The Costa reaction is a very simple test which can be carried out in twenty minutes and does not necessitate venipuncture. Its purposes may be compared to those of the sedimentation test.
2. The test was carried out in 200 cases which included a variety of pathologic conditions.
3. In active pulmonary tuberculosis the test is positive in a high percentage of cases.
4. Ninety per cent of the cases of proved malignancy were Costa positive.
5. Syphilis apparently has no influence on the reaction.
6. Fever in itself does not give a positive test.

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## THE HETEROPHILE ANTIBODY REACTION IN THE DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS\*

E. M. BUTT, M.D., AND A. G. FOORD, M.D., PASADENA, CALIF.

**D**URING the course of a study on heterophile antibodies, demonstrable in the form of sheep cell agglutinins and hemolysins, in a variety of clinical conditions, Paul and Bunnell<sup>1</sup> found that high titers of such antibodies were present in the blood sera of cases of infectious mononucleosis. Previous to the appearance of this article, Davidsohn<sup>2, 3</sup> had demonstrated the presence of similar agglutinins in cases of serum sickness. Later Bunnell<sup>4</sup> reported fifteen additional cases of infectious mononucleosis and called attention to the diagnostic value of the test in this disease. In this latter series of cases, sheep cell agglutinins were obtained in titers varying from 1 to 64, to 1 to 4,096. These investigators found that the titers of heterophile antibodies in normal human cases rarely exceeded a dilution of 1 to 8. They further reported no increase in agglutinins above the normal in a variety of clinical conditions other than infectious mononucleosis and serum sickness. Sprunt,<sup>5</sup> Rosenthal and Wenkeback,<sup>6</sup> and Olesen<sup>7</sup> have corroborated Paul and Bunnell's findings in acute mononucleosis. For a complete discussion of the heterophile antibodies we refer the reader to an extensive summary by Davidsohn.<sup>8</sup>

**Methods.**—We have not varied the original technique described by Paul and Bunnell,<sup>1</sup> which is as follows: The sera were obtained as for a Wassermann test, inactivated for fifteen minutes at 55° C., and diluted from 1 to 4, to 1 to 32 or higher, if necessary, in 0.5 c.c. portions with normal saline. To each tube containing the diluted sera, 0.5 c.c. of a 2 per cent suspension of sheep cells and 1.0 c.c. of salt solution were added. The test tubes were

\*From the University of Southern California School of Medicine, the Santa Fe Coast Lines and Pasadena Hospitals.

Received for publication, July 12, 1934.



shaken, placed in the water-bath at 37° for one hour and left in the ice box overnight. The following morning the readings were made. The following symbols were used in the quantitation of the agglutinins:

- +++ = Firm disk
- ++ = Disk easily broken into large flakes
- + = Fine
- ± = Barely perceptible, but definite agglutination

In addition to this procedure, we have added to our routine tests a microscopic agglutination technic for quick diagnosis. This consists of taking one loopful of blood serum to be tested and four loopfuls of a 2 per cent suspension of sheep cells in normal saline, and making a hang-drop preparation. Almost immediate agglutination takes place when sera from cases of infectious mononucleosis and serum disease are employed. Sera from other conditions are negative by this test even though by the test tube agglutination method low titers of sheep cell agglutinins are demonstrable.

Hemolysin and agglutinin tests were run simultaneously in the beginning but as the results were so nearly alike, we discontinued the hemolysin test. Hemolysins were demonstrated by adding an excess of guinea pig complement to the diluted, inactivated human sera, together with sheep cells and placing in a water-bath at 37° for one hour.

Sporadic cases of infectious mononucleosis are not uncommon, in fact we are of the opinion that if the ubiquitous infections, commonly called "colds," "influenza" or "sore throats" were studied, many more cases of this disease would be recognized. From our hospital services and the practices of some of our medical associates, we have been able to collect 18 cases of infectious mononucleosis during the past seven months. Regarding the diagnostic aspects of these cases, suffice it to say that the symptoms, the clinical course of the disease, and the laboratory findings were classically diagnostic of the disease. Sheep cell agglutinins were found in titers varying from 1 to 64, to 1 to 2,048 in seventeen of the eighteen cases. These results are compiled in Table I. The serum from one patient (Case 2, Table I), a young woman who had had the disease seventeen months prior to the performance of the test, contained no heterophile antibodies. She ran the typical clinical course and blood studies at that time indicated that she undoubtedly had infectious mononucleosis.

In addition to the foregoing cases we tested the sera of 412 hospitalized adult patients for sheep cell agglutinins. This group includes a variety of conditions too numerous to cite, which may roughly be classed as infections, chronic diseases, blood dyscrasias and injuries. No sheep cell agglutinins were present in 81 per cent of the sera in dilutions of 1 to 4 or higher. The titer of agglutinins in the remaining 19 per cent did not exceed a serum dilution of 1 to 8 except in one case in which the antibodies were present in a 1 to 16 dilution of the serum. These facts will be found in Table II.

The sheep cell agglutinin studies of a few conditions thought to be of pertinent interest in the evaluation of this diagnostic test are summarized in Table III. Heterophile antibodies were not found in nine cases of leucemia

TABLE I  
CASES OF INFECTIOUS MONONUCLEOSIS

CASE	SEX	AGE	OCCUPATION	ELAPSED TIME IN WEEKS BE- TWEEN DATE OF ONSET OF DISEASE AND PER- FORMANCE OF TEST	W.B.C.	PER CENT LYMPHO- CYTES INCLUDING ATYPICAL FORMS	SHEEP CELL AGGLUTININS, DILUTION OF PATIENT'S SERUM									
							1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048
1	Female	22	Nurse	12	7,600	70	+++	++	+	±	±	-	-	-	-	-
2	Female	25	Housewife	17 (mo.)	9,700	76	--	--	--	--	--	--	--	--	--	--
3	Male	26	Engineer	3	12,000	71	+++	+++	+++	+	±	±	-	-	-	-
4	Female	25	Housewife	6	8,900	72	+++	+++	+++	+++	+	++	+	±	-	-
5	Female	20	Student	6	8,300	51	+++	+++	+++	±	±	-	-	-	-	-
6	Male	19	Student	2 (days)	12,800	60	+++	+++	+++	+++	+++	+	±	-	-	-
				7 (days)	18,800	84	+++	+++	+++	+++	+++	++	++	+	±	-
7	Male			6	9,000	49	+++	+++	+++	±	±	-	-	-	-	-
8	Female	30	Nurse	6	7,400	72	+++	+++	+	+	±	-	-	-	-	-
9	Male	34	Physician	2	14,000	65	+++	+++	+++	+	±	-	-	-	-	-
				4	20,000	67	Serum not received									
10	Male	18	Student	4	9,600	70	+++	+++	+++	+	±	-	-	-	-	-
				6	8,050	58	+++	+++	+	±	±	-	-	-	-	-
				4	14,000	50	+++	+++	+++	±	±	-	-	-	-	-
11	Male			6	-----	--	+++	+++	--	--	--	--	--	--	--	--
12	Female	17	Student	1	20,000	82.5	+++	+++	+++	+++	+++	+++	++	++	+	±
13	Male	18	Student	5 (days)	15,600	85	+++	+++	+++	+++	+++	+	±	-	-	-
				4 (days)	18,000	88	+++	+++	+++	+++	+++	+	±	-	-	-
14	Male	22	Medical student	4	-----	--	Serum not received									
				5 (days)	24,000	77	+++	+++	+++	+	+	++	±	±	-	-
				3 1/2	7,600	73	+++	+++	+++	+++	+++	+	±	-	-	-
				8 1/2	6,200	52	+++	+++	+++	+	+	+	-	-	-	-
15	Male	22	Secretary	12 1/2	5,500	49	+++	+++	±	-	-	-	-	-	-	-
16	Male	25	Printer	5 (days)	17,000	60	+++	+++	+++	+++	+++	++	+	±	-	-
17	Male	35	Physician	10 (days)	12,680	80	+++	+++	+++	+	±	++	-	-	-	-
				10 (days)	14,300	80	+++	+++	+++	+	+	±	-	-	-	-
18	Male	21	Student	8	-----	--	+++	+++	+++	±	±	-	-	-	-	-
				7 (days)	10,000	70	+++	+++	+++	+++	+++	±	±	-	-	-
				9 (days)	7,200	72	+++	+++	+++	+++	+++	+	±	-	-	-

TABLE II  
SHEEP CELL AGGLUTININS IN A VARIETY OF CONTROL CONDITIONS SUCH AS INFECTIONS, CHRONIC DISEASES, AND INJURIES

NUMBER OF CASES	PER CENT	SHEEP CELL AGGLUTININS, DILUTION OF SERUM			
		$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$
336	81.7	---	---	---	---
43	10.4	±	---	---	---
9	2.2	+	---	---	---
2	0.5	++	---	---	---
8	1.9	+	±	---	---
5	1.2	++	±	---	---
6	1.4	++	+	---	---
2	0.5	++	++	---	---
1	0.2	+++	+	±	---
412					

TABLE III

SHEEP CELL AGGLUTININS IN CASES OF IMPORTANCE FOR THE EVALUATION OF THE TEST FOR THE DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS

DISEASE	NUMBER OF CASES	SHEEP CELL AGGLUTININS DILUTION OF SERUM				
		$\frac{1}{4}$	$\frac{1}{8}$	$\frac{1}{16}$	$\frac{1}{32}$	$\frac{1}{64}$
Lymphatic leucemia	5	---	---	---	---	---
Myelogenous leucemia	4	---	---	---	---	---
Aplastic and pernicious anemia	2	---	---	---	---	---
Hodgkin's disease	1	---	---	---	---	---
Myeloma	1	---	---	---	---	---
Hay fever	3	---	---	---	---	---
Urticaria	2	---	---	---	---	---
Asthma	2	---	---	---	---	---
Electric burn	1	+++	+++	++	±	---
Serum disease	2 (1)	+++	+++	++	+	±
	(2)	++	++	±	---	---
Paratyphoid B. infections	2	±	---	---	---	---
Bacillary dysentery (Flexneri)	1	±	---	---	---	---

and one case each of aplastic and pernicious anemia. This fact greatly augments the value of the heterophile agglutinin test in infectious mononucleosis, a disease that has been confused in the early stages with lymphatic leucemia.

Attention is called to two cases of food poisoning due to *B. paratyphosus* B, which are of interest in view of Iijima's<sup>9</sup> findings that strains of these organisms contain the heterophile antigen. The patients had severe diarrheas lasting approximately three days and terminating in complete recovery in about five days. The agglutination test did not become positive and the sheep cell agglutinins were present only in small quantities in the 1 to 4 dilutions of the sera.

Of particular interest in this series of cases is that of a young adult who sustained severe electrical burns of the hands and arms. His blood serum contained a rather high titer of sheep cell agglutinins. The patient had been quite well previous to his accident, and careful examination while in the hospital failed to reveal any symptoms or signs diagnostic of infectious mononucleosis. He had not received horse serum, but shortly after his entrance into the hospital, he developed a generalized urticarial type of lesion of the skin.

suggestive of some allergic phenomenon. A short time later the patient passed out of our control, preventing further observations.

In view of the fact that Taniguchi<sup>10</sup> has pointed out the possibility of an error in the Wassermann reaction using sera that are rich in sheep cell hemolysins, we performed Wassermann tests on our cases of infectious mononucleosis and serum disease. To our surprise, and contrary to their suggestion, we found that high concentration of heterophile antibodies did not affect our complement fixation test, which was negative in the cases of infectious mononucleosis and serum disease. Furthermore, a nonspecific fixation of complement was not obtained in any of the control cases listed in Table II. The reason for this is not clear, but it may be due to the absence of heterophile antigens in the beef heart preparations that we use in the Wassermann reaction, for it is a well-known fact that fixation of complement will occur in heterophile antigen and antibody reactions.

#### CONCLUSION

Corroboratory evidence is offered in support of Paul and Bunnell's test for infectious mononucleosis. The points of interest are, that the presence of heterophile antibodies in high concentration is a constant feature of the disease; that the production of heterophile agglutinins is roughly parallel to the leucocytosis; and further, that the height of the agglutinin response is dependent upon the stage of the disease in which the serum is obtained for testing.

A nonspecific fixation of complement was not obtained in the Wassermann test with sera containing high concentrations of heterophile antibodies.

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# A NEW SLIDE AND HAND SHAKER FOR USE IN SLIDE PRECIPITATION AND AGGLUTINATION STUDIES IN IMMUNOLOGY\*

B. S. LEVINE, PH.D., CHICAGO, ILL.

THE use of the glass slide and the paraffin ring method in the precipitation and agglutination procedures for the laboratory diagnosis of diseases is now being widely resorted to in the fields of bacteriology and serology. The latest application of the slide precipitation procedure is represented by the

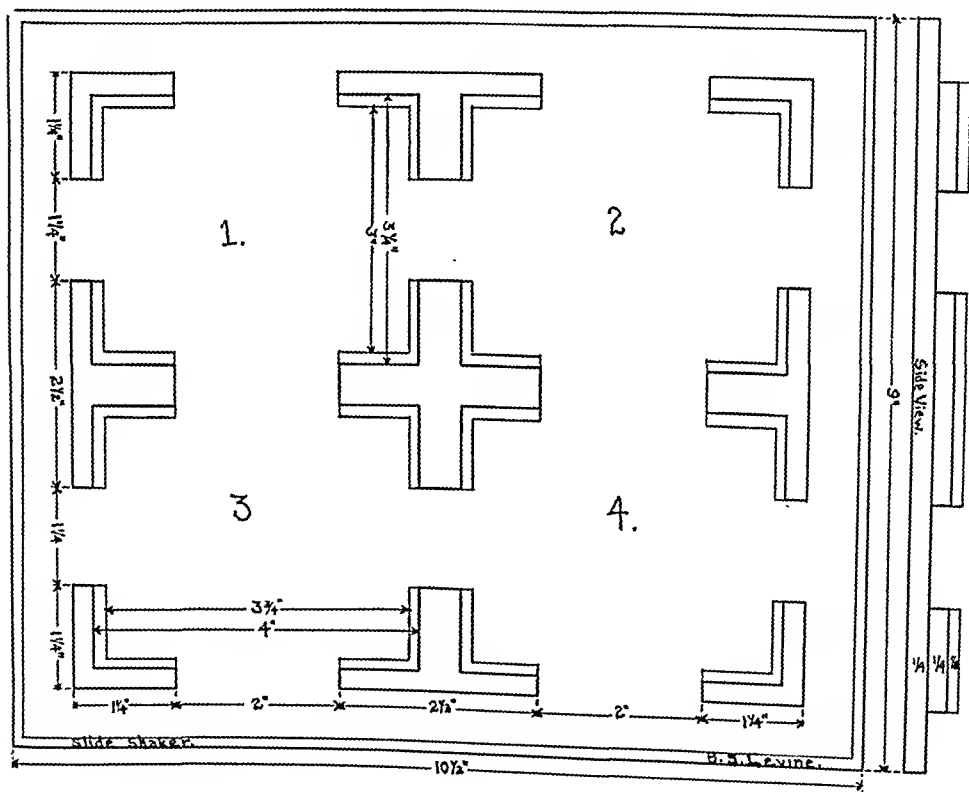


Fig. 1.

slide method of Kline.<sup>1</sup> He recommends the use of glass slides 2 inches by 3 inches upon which a maximum of 12 paraffin rings can be made. The slides are agitated individually by hand, or the Magath mechanical shaker<sup>2</sup> can be used.

The equipment described here simplifies the procedure of hand shaking and reduces the cost of the shaker to a nominal sum. The slide is the same

\*From the Clinical Laboratory of the Public Health Institute.  
Received for publication, July 6, 1934.

as is ordinarily used in the preparation of lantern slides and measures 3 inches by 4 inches. Depending upon the size of the paraffin rings desired, from 20 to 25 agglutination or precipitation tests can be performed with each slide at one time. This size of the slide is such as to make its use conveniently adapted to any standard microscope. The shaker is made of fiber, wood, plywood, celluloid, or any other board material about  $\frac{1}{4}$  inch thick. It can be easily constructed by following the instructions schematically presented in Fig. 1.

The paraffin rings are made upon the glass slides as usual. The slides are then numbered from 1 to 4 and placed into the corresponding sockets of the shaker. The serums and the antigen are now deposited within the rings in the required volumes and order, in accordance with the particular immunodiagnostic procedure that is being used. The shaking process can be

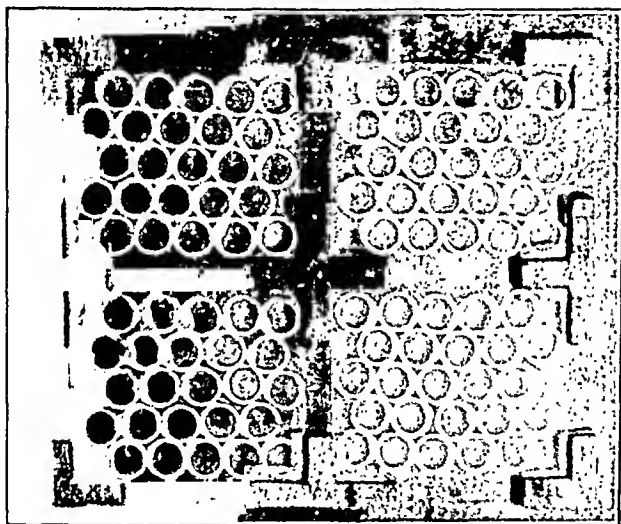


Fig. 2.

accomplished by resting the shaker upon the laboratory table and imparting it a one-plane rotatory movement by hand. The shaker can also be held in the palms of both hands and agitating it so that its four corners move up and down in a rapid successive order, while its center remains stationary, as though it were resting atop a sharp pivot. Both types of agitation movement can be used alternately several times, if it is found more expedient and effective a mixing procedure.

From 80 to 100 agglutination or precipitation tests can thus be performed simultaneously within a short time. To prevent evaporation due to prolonged exposure of the slides to the air, two laboratory workers may divide the work so that while one is preparing specimens labeled from 1 to 50, the other may be working on specimens labeled from 51 to 100. The final set-up is shown in Fig. 2. When the shaking is completed, a similar division of work can be profitably resorted to in making the microscopic examinations and in the recording of the final results.

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## A STABLE, STANDARDIZED COLLOIDAL GOLD SOLUTION\*

WILLIAM C. WILLIAMS, WASHINGTON, D. C.

THE colloidal gold test introduced by Lange<sup>1</sup> in 1913 is still recognized as one of the most valuable aids available for the diagnosis and prognosis of syphilis of the central nervous system. However, most laboratory workers have experienced considerable difficulty, at one time or another, in the routine preparation of a uniformly satisfactory colloidal gold solution. Consequently, many modifications of the original method have been proposed in an endeavor to overcome some of the difficulties. No definite standard for gold sol has existed; usually a rough test for precipitability by a 1 per cent salt solution has sufficed and it was only rarely that two gold sols could be prepared which were identical in sensitivity.

In undertaking certain investigations on the colloidal gold reaction with various globulins and globulin fractions, it was essential that results over a considerable period of time be comparable. To insure this, some method of preparation resulting in a gold sol of the same character and sensitiveness as all previous lots prepared, i.e., a *standard* gold sol had to be found. So far as known, none of the methods previously advocated consistently furnished uniform, standard solutions, each lot of which could be made identical with all previous lots.

The method to be described is adapted from a procedure proposed by Patterson,<sup>2,3</sup> in 1931, which offered the following advantages: (a) A definite titration of reagents before the stock solution is made; (b) an alkaline stock solution which is relatively stable; (c) a second titration against a globulin to determine the exact amount of hydrochloric acid which should be added just before use; (d) the ability by (c) to exactly adjust each lot of colloidal gold solution so that it is identical in intensity and sensitivity with all previous lots made.

With the adoption of certain modifications, here described, gold sol prepared and titrated by this method has proved highly satisfactory and has given excellent results for a period of over two years, during which time more than 1,500 spinal fluids have been tested. Patterson's method will not be quoted in detail here, only the changes and modifications being noted. For

\*From the Division of Serology, Department of Laboratories, Army Medical School, Army Medical Center.

Received for publication, July 9, 1934.

the original technic the reader is referred to his article<sup>2</sup> or the abstract.<sup>3</sup> Some of the changes were made because of the availability of reagents but other important modifications in the titrations were made because they appear to result in a distinct improvement of the method and the final product. Briefly, these changes are as follows: (a) The use of gold chloride, acid salt, Merek's Blue label or equivalent, instead of gold sodium chloride, double salt; (b) the use of 0.02 N potassium hydroxide solution instead of sodium hydroxide; (c) the direct measurement of the potassium hydroxide solution into the tubes of the preliminary titration, instead of the use of the drop method; (d) the use of a solution of edestin, a vegetable globulin from hemp seed, first used for this purpose by Kriedler and Small,<sup>4</sup> for the final titration, instead of the solution of hemoglobin used by Patterson.

The details of the technic developed and used in this laboratory are as follows:

#### REAGENTS

1. *Distilled Water*.—Double distilled water is preferable, although satisfactory gold sols are being made with freshly distilled water from a good still.

2. *Gold Chloride*.—(Merek's blue label or equivalent) 1 per cent solution. This solution is made in the usual way by breaking the ampule inside of a hard glass bottle and adding the required amount of distilled water.

3. *Potassium Oxalate*.—(Highest purity) one per cent solution. Must be freshly prepared and should not be cloudy.

4. *Potassium hydroxide Solution 0.02 N*.

5. *Hydrochloric Acid, 0.02 N*.

6. *Edestin*.—(Highest purity, Pfanstiehl.) One to 2,000 solution: Since edestin is insoluble in water or weak salt solutions, 0.2 per cent hydrochloric acid is used as the solvent. This amount of HCl does not affect the final titration.

*Glassware*.—Tubes, beakers, pipettes, Florence flasks, bottles for solutions, etc., as required. All glassware should be of high grade resistant glass, chemically clean but not necessarily sterile. It should be washed with aqua regia, followed by seven or eight rinsings in tap water, with a final rinsing with distilled water.

#### PRELIMINARY TITRATION

Nine, dry, chemically clean test tubes are placed in a rack and numbered. To the bottom of these tubes, pipette increasing amounts of 0.02 N potassium hydroxide solution as follows: No. 1, none; No. 2, 0.15 c.c.; No. 3, 0.2 c.c.; No. 4, 0.25 c.c.; No. 5, 0.3 c.c.; No. 6, 0.35 c.c.; No. 7, 0.4 c.c.; No. 8, 0.45 c.c.; and No. 9, 0.5 c.c. Next, place in a small beaker or flask, 50 c.c. of distilled water, 0.5 c.c. of 1 per cent potassium oxalate solution, and 0.5 c.c. of 1 per cent gold chloride solution. Mix and add 5.0 c.c. of this solution to each of the nine tubes. Immediately immerse the tubes in a beaker containing sufficient water, at room temperature, to just cover the level of the fluid in the tubes. Place the beaker, protected by plain wire gauze, over a Bunsen burner,



rapidly bring the water to the boiling point and boil for two minutes. Withdraw the tubes and place them in order in the rack. Only one tube in the series represents the right amount of potassium hydroxide to use in the preparation of the stock gold sol. It is the lowest tube in the series to give a bright red solution, which, when viewed by reflected light, shows just the slightest sheen. The other tubes are eliminated by being too purple, too pale or even colorless. A simple multiplication by 200 gives the correct amount of 0.02 N potassium hydroxide to use in the preparation of 1,000 c.c. of stock gold sol.

#### PREPARATION OF THE STOCK GOLD SOL

As the stock colloidal gold solution is perfectly stable, the amount made is only limited by the requirements of the laboratory and by the quantity of reagents originally prepared. A new preliminary titration is necessary whenever new reagents are made. In this laboratory it has been found convenient to prepare eight or more individual lots of 1,000 c.c. each, which are pooled and kept in a resistant glass bottle.

To prepare 1,000 c.c. a three liter Florence flask is used. Place 1,000 c.c. of distilled water in the flask, add 10 c.c. of 1 per cent oxalate solution, 10 c.c. of 1 per cent gold chloride solution, and the amount of 0.02 N potassium hydroxide solution determined by the preliminary titration, which is, usually, between 40 and 50 c.c. Bring to the boiling point rapidly, without shaking. The solution will go through the various color changes from colorless to pale blue, blue, purple, and, finally, a very deep dark red. Just before the boiling point is reached there is a sudden "*lightening*" of the solution, which becomes a clear bright red color. No further color change takes place, although the solution should be left over the flame until boiling starts. This "*lightening*" of the mixture is an *absolute essential* to a satisfactory solution. If this typical change does not occur, there is something wrong with the reagents, their measurement, or the calculation of the amount of potassium hydroxide used. After the various lots are pooled and have stood overnight, the stock solution is ready for the final titration.

#### FINAL TITRATION OF THE STOCK SOLUTION

Inasmuch as the stock solution prepared according to this method is alkaline, it is necessary to determine the amount of hydrochloric acid to be added to any portion of it, just before use. This is done in the following manner: Six series of ten tubes each are set up exactly as for the routine test on spinal fluid except that smaller tubes, 12 by 120 mm. are satisfactory, since only one-half the usual quantities are used. To the first tube in each series add 0.9 c.c. of 0.4 per cent salt solution and to each of the other tubes 0.5 c.c. Prepare a 1 to 2,000 solution of edestin in 0.2 per cent hydrochloric acid and place 0.1 c.c. of this solution in the first tube of each series. Make dilutions in the usual way by mixing thoroughly the contents of the first tube and carrying over 0.5 c.c. to the second tube, mixing, and so on in series to the tenth tube, from which 0.5 c.c. of the mixture is discarded.

Six 25 c.c. portions of the stock gold sol are placed in small 50 c.c. flasks and numbered. These are acidified with 0.02 N HCl as shown in Table I.

TABLE I

FLASK	STOCK SOLUTION	0.02 N HCl
1	25 c.c.	0.25 c.c.
2	25 c.c.	0.30 c.c.
3	25 c.c.	0.35 c.c.
4	25 c.c.	0.40 c.c.
5	25 c.c.	0.45 c.c.
6	25 c.c.	0.50 c.c.

Each tube of Series 1 then receives 2.5 c.c. of acidified Sol 1; each tube of Series 2, 2.5 c.c. of acidified Sol 2 and so on until the six series have received the six specimens of acidified gold sol. The tubes are mixed by gentle shaking or by rotating the individual tubes between the hands. Results are read after eighteen to twenty-four hours.

Various degrees of reaction will be evident in the different series. That amount of 0.02 N HCl which just gives a moderately strong "paretic" type of reaction (5555+21000) is the correct amount to use in acidifying the stock gold sol just before use. Usually this is found in the third or fourth series, containing 0.35 or 0.4 c.c. of the acid per 25 c.c. of stock sol, but may vary considerably with different preparations. The stock bottle is labelled with the result of the titration.

Before a gold sol is adjudged satisfactory and placed in routine use, it should be tested several times with known normal and paretic spinal fluids. It has been our experience that a colloidal gold solution giving a satisfactory reaction with the spinal fluid of a paretic will also give typical reactions with fluids from patients with other types of syphilitic involvement of the central nervous system. As suggested by Kriedler and Small,<sup>4</sup> specimens of spinal fluid may be preserved from eight to ten weeks by adding an equal quantity of pure glycerin. This does not affect the colloidal gold reaction. Twice the amount of spinal fluid-glycerin mixture, of course, should be used in testing. The use of a fresh or preserved paretic spinal fluid in the final titration of the stock solution to determine the amount of 0.02 N HCl necessary, is perfectly feasible. The advantage of the edestin solution lies in the fact that a definite strength globulin solution may be prepared whenever needed.

#### DISCUSSION

The advantages of an easily prepared colloidal gold solution, which is stable and in which each lot can be made identical in sensitivity with all previous lots, needs no emphasis.

The difficulties so frequently encountered when using the formalin reduction methods, occur without apparent reason, even when using the reagents and distilled water with which previous satisfactory solutions have been prepared. These difficulties may persist for some time, disappearing suddenly, again for no apparent reason. No definite standard has existed for

gold sol, and it has been but rarely that two gold sols could be produced which were identical in sensitivity. Colloidal gold solutions have been unstable; a precipitate, forming after a period of a few weeks, renders the solution unfit for use.

The method suggested by Patterson seemed to obviate some of these difficulties and was given an extended trial. Using the original technic, certain anomalous results were obtained, requiring the modifications noted. The changes were made for reasons of increased accuracy, availability of certain reagents and definite improvement in the standardization of the final product.

In the preliminary titration, direct measurement of the hydroxide into the dry tube was adopted, instead of the drop method, because of its accuracy, simplicity and the ease of calculation of the total amount required in preparing the stock solution.

The substitution of edestin for Patterson's solution of hemoglobin was adopted for greater accuracy in the final titration. While usable gold sol could be made when final adjustment was done with a 1 per cent solution of hemoglobin, it was felt that this was the weak point in the procedure. The difficulty and inaccuracy of making an exact 1 per cent solution of hemoglobin from the few drops of blood which Patterson used, is manifest. Larger amounts of packed cells were used with somewhat better results but a new gold sol was not necessarily identical with a previous gold sol which had been titrated against an entirely different hemoglobin solution. Edestin can be accurately weighed and solutions of definite strengths made. It was found that a 1 to 2,000 solution of edestin gave a typical "paretic curve" and was entirely satisfactory as an indicator by which the sol could be adjusted so that it was exactly comparable with all previous lots made.

The technic as given, which has been in use for over two years, has resulted in consistently good colloidal gold solutions. The stock solution is stable, allowing large quantities to be prepared at one time. The titrations are simple and the amount of 0.02 N HCl required by any given quantity prior to use, does not change during the life of the stock solution.

#### SUMMARY

A method of preparing a stable, standardized colloidal gold solution, which has given excellent results during an extended trial, is presented in detail.

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## CLINICAL PHOTOGRAPHY WITH THE LEICA CAMERA\*

WARREN T. VAUGHAN, M.D., RICHMOND, VA.

I HAVE been searching for some time for a satisfactory camera with which to visually record interesting clinical observations. Scarcely even a novice in photography, I tried out several very excellent cameras but found them unsatisfactory for my purpose and too expensive when it came to commercial finishing and printing. At the Milwaukee meeting of The American Medical



Fig. 1.—Intracutaneous sensitization tests applied to the back. An average size indoor shot with F 3.5 lens (50 mm.) at three and one-half feet. Exposure 1/40 second. Source of light: daylight and photoflood. Film: Eastman Supersensitive Panchromatic.

Association I observed two physicians with small cameras apparently no larger than the vest pocket size, taking snapshots in the subdued light of the scientific exhibit. Feeling that, if these photographers actually had anything on the

\*Received for publication, July 31, 1934.

film when they returned home, it would be a very desirable type of camera, I inquired as to the make. The result was the purchase of a Leica Camera made by E. Leitz, Inc., well known as manufacturers of microscopes and lenses. Trouble at once began. It was soon found that one must know



Fig. 2.—Tuberculosis cutis. Elmar 50 mm. lens with front lens No. 2, 21½ inches from object. Stop 4.5. Source of light: daylight and photo flood. "B" filter. Exposure 1/20 second. Film S. S. Pan.



Fig. 3.—Detail of teeth. 50 mm. Elmar lens. No. 3 front lens. Distance 12¼ inches. No filter. Stop 6.3. Time 1/20 second. Source of light: daylight and one photo flood. Film S.S. Panchromatic. Print prepared for dentist who wishes to make an artificial denture resembling the patient's original teeth as closely as possible. The illustration shows pyorrhea, recession, and thickening of the gums unusually well.

something of photography to use a precision instrument, otherwise one would be better off pressing the button of a box Brownie. I was very fortunate in being able to procure the services of Mr. John P. Tillery, an expert in pho-

tography, who in a remarkably short period of time instructed me in the intricacies of really expert photography.\*

The details of technic having been once mastered, the flexibility and range of usefulness of this camera is truly astonishing. It is so small that it



Fig. 1.—Acute trichophytosis of hand with secondary infection and extensive tissue destruction. Much of the dead skin has been trimmed away. Elmar 50 mm. lens and sliding focusing copy apparatus. Green "B" (58) filter. Exposure 1/30 second. This picture forms an excellent record for comparison after treatment. The hand is now completely cured.



Fig. 5.—Close-up showing detail of eye. Elmar 50 mm. lens with front lens No. 3. No filter. Snapshot at 1/40 second using Supersensitive Panchromatic film.

may be carried in the pocket, and yet with the appropriate accessory attachments it can be used for anything from photomicrography at one extreme to telephotography at the other. The modern photoflood lamp makes indoor snapshots as easy as or even easier than outdoor shots. The so-called speed lenses make night photography simple. The fine quality of the lenses and

\*Mr. Tillery's services are available for this purpose.

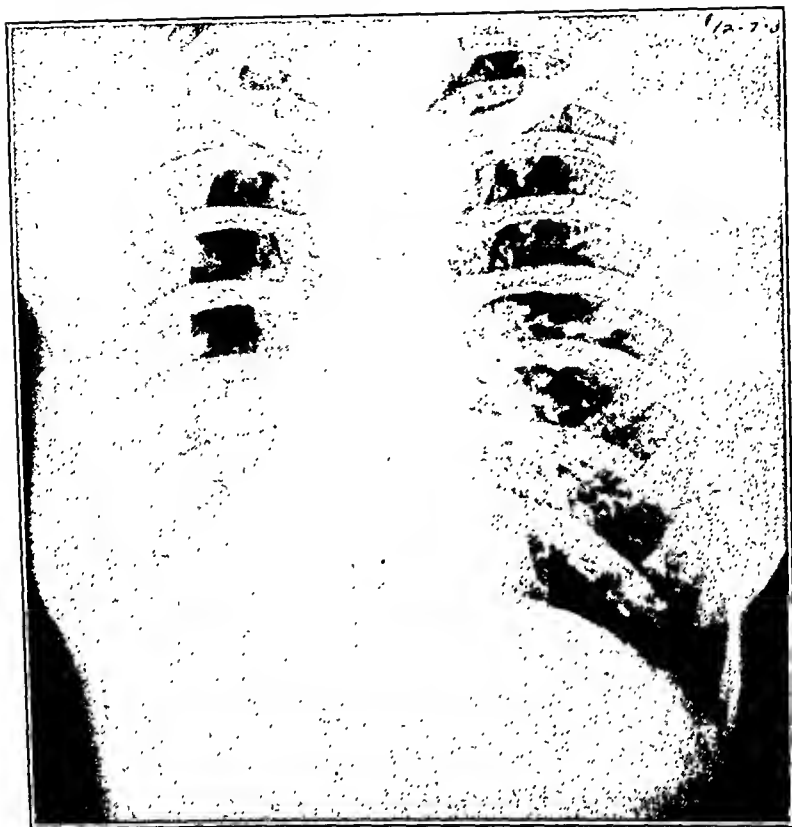


Fig. 6.—Photograph of x-ray film exposed in ordinary x-ray viewing box. Time one second at F 6.3. Source of light: the illumination of the viewing box. 50 mm. Elmar lens. Film, S. S. Pan.

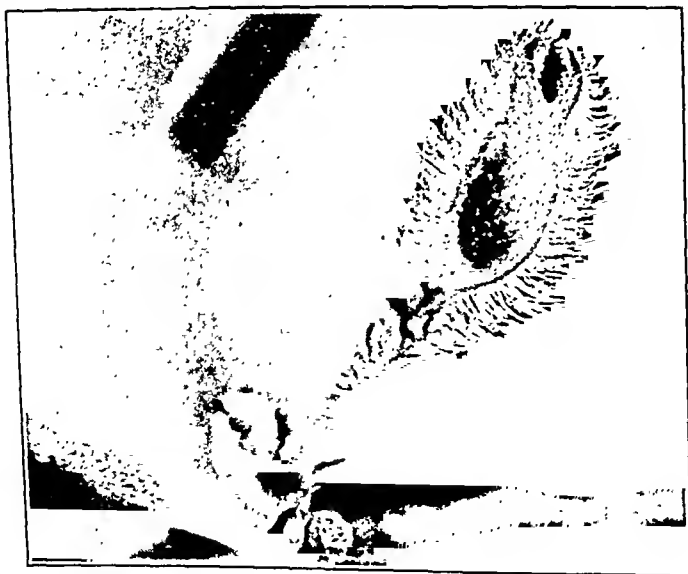
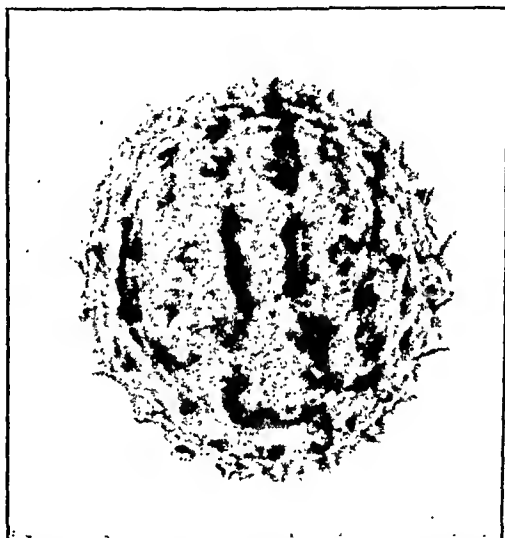
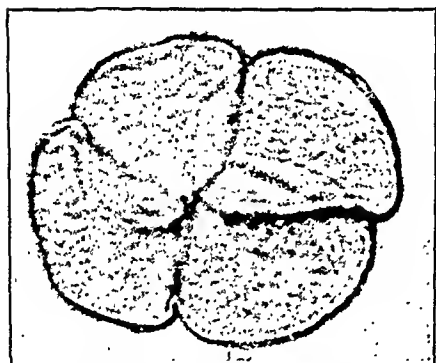


Fig. 7.—Photograph of elm bud made with sliding focusing copy apparatus on plena-chrome film with the source of light 2 photoflood bulbs and the time  $1/20$  second.

of the motion picture film which is used in the camera permit enlargement ten or more times without the appearance of grain.

Although using motion picture film the Leica is a "still" camera. From 36 to 40 exposures may be made on one loading. This amount of film costs about twenty-five cents when purchased in bulk. After the initial investment the cost of photography is reduced to a minimum, since we are dealing with a miniature camera requiring only a very small amount of film surface and of developing material. And yet, when these exposures measuring an inch by an inch and one-half, scarcely larger than a postage stamp, are enlarged, they are as clear as are contact prints made with larger cameras.

Since the camera is so small and can be easily carried in the pocket, its versatility is still further increased in that it may be used for pleasure as well as in one's work.



Figs. 8 and 9.—Photomicrographs of oat-tall and dandelion pollen, enlarged one thousand times. Spencer microscope with high dry objective, No. 10 eye piece, sliding focusing copy apparatus with extension tubes and no camera lens. Source of light: one photoflood bulb. Exposure three seconds, using plenachrome film.

The nine accompanying photographs, from the writer's collection, illustrate the flexibility and versatility of the Leica in clinical work.

Charts and other illustrations may be photographed. Lantern slides are prepared without difficulty. Figs. 7 to 9 illustrate the flexibility of the apparatus.

From the illustrations it will be seen that passable clinical photographs may be made by a novice. The average clinician has neither time nor room in his offices for an elaborate photographic set-up. Speed lenses, supersensitive film, and the inexpensive photoflood bulbs permit of snapshots indoors. This obviates the necessity of an elaborate set-up and enables one within five minutes to have made a permanent record of any interesting or unusual clinical observation. The inexpensiveness of the film (less than one cent per shot) makes it possible to keep permanent records of any type of clinical subject which is photographically recordable.



# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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**RETICULOCTE**, Count in Healthy Children, Osgood, E. E., Baker, R. L. and Wilhelm, M. M. *Am. J. Clin. Path.* 4: 292, 1934.

Reticuloocyte counts in 208 healthy children, about equally distributed as to sex and age between the ages of four and thirteen, inclusive, averaged 1.47 per cent or 71,473 per c.mm. and ranged from 0.4 to 3.8 per cent or from 18,640 to 184,800 per c.mm.

There were no significant variations in either the absolute or percentage counts for the different sex and age groups.

There were no significant variations in the percentage counts from the values previously found for men and women.

Reticuloocyte counts should be reported in percentage rather than in absolute figures.

Normal values for reticuloocyte percentage in adults or in children between the ages of four and thirteen, inclusive, are an average of 1.5 per cent with a range of 0.5 to 3.8 per cent, when a method giving optimum conditions for reticuloocyte staining is used.

**MILK**, Effect of Temperature of Incubation Upon Agar Plate Count of, Pederson, C. S., and Yale, M. W. *Am. J. Pub. Health* 24: 477, 1934.

An incubation temperature of 32° C. for forty-eight hours instead of 37° C. is recommended for standard agar plates prepared from samples of milk and ice cream.

With forty-eight-hour incubation, higher colony counts are obtained at 32° C. than at 37° C., resulting in a truer measure of quality.

At 32° C. there is less error in counts than at 37° C. due to temperature variation in the incubator.

The percentage of the maximum counts obtained in forty-eight hours varies considerably at 37° C. Therefore counts obtained at the latter temperature serve as a better means of comparing the quality of different samples.

**"CARRIERS,"** Detection of, Among Food Handlers in Connecticut, West, D. E., Borman, E. K., and Mickle, F. L. *Am. J. Pub. Health* 24: 493, 1934.

Statistics on laboratory examinations of milk-handler specimens over a period of seventy-seven months and on other food-handler specimens covering a period of thirty-two months are presented in a manner to show the total number of examinations, the number of positive examinations, and the number of individuals represented by the positive examinations for each year and type of examination.

Statistics on 91,257 laboratory examinations of milk-handler specimens are subjected to an analysis showing estimates of the annual costs based on a unit cost per examination varying slightly from year to year. Significant figures brought forth are: total cost of the 91,257 examinations in seventy-seven months, \$48,048; average cost of detecting each of the 71 carriers found, \$677; per capita cost for 1932, less than  $\frac{2}{3}$  cent; cost per consumer per annum less than 2 cents in 1933; cost per 1,000 quarts of milk (all grades), less than 7 cents in 1933. The cost per carrier per annum was found to vary from year to year, from a minimum of \$387 to a maximum of \$3,848.

A correlation of results obtained with the funds expended establishes the general utility and importance of routine laboratory examinations made periodically on important groups of food handlers in a central laboratory doing a large volume of work.

**TUBERCULOSIS: A Successful Method of Cultivating Tubercle Bacilli, Cowen, M. E., and Henderson, E. J. Am. Rev. Tuberc. 29: 368, 1934.**

The medium used is prepared as follows (formula for about 120 tubes):

450 c.c. milk (cream removed)

18 gm. potato flour

2.6 gm. asparagin

225 gm. potato (peeled and sliced thin)

12 eggs

3 egg yolks (additional)

35 c.c. glycerine C.P. (sterile)

30 c.c. 2 per cent aqueous solution of malachite green

Put the sliced potato, the potato flour and the milk into a double boiler and dissolve the asparagin in the mixture. Cook for two hours, stirring constantly until the mixture becomes sticky; then stir occasionally.

Sterilize the eggs by rubbing with alcohol and flaming. Break the whole eggs into a sterile 2-liter Erlenmeyer flask, add the extra egg yolks and shake well, breaking the yolks with a sterile glass rod if necessary. Use a sterile cork or rubber stopper in the flask while shaking. Add the glycerine and the dye solution and shake again.

Cool the potato-milk mixture to 45-50° C.; add the egg-glycerine mixture slowly, stirring thoroughly; and filter through sterile gauze into a sterile beaker. The medium thus prepared is neutral to litmus.

The shaking of the flask causes a very persistent, fine-bubbled foam, which should be removed by skimming with filter paper or with a sterilized piece of fine wire gauze; otherwise the slants will have an undesirable porous surface. The medium, in 4 to 5 c.c. quantity, as desired, is now poured into culture tubes which have been sterilized with their cotton plugs inserted. The cotton plugs are replaced and covered with rubber caps instead of with paraffin.

The final sterilization of the tubed medium is in an Arnold sterilizer. The tubes are arranged in a slanting position in the cold sterilizer, and care must be taken that they do not slant enough to bring the medium into contact with the cotton plugs.

The sterilizer is heated slowly to 80° C. which should require about one and one-half hours, and the temperature should not be permitted to go much beyond that point. It can be controlled easily by the opening and closing of the sterilizer door.

It is preferred not to let the temperature get above 45° during the first half-hour and from 45 to 65° in the second half-hour; reach 80° at the end of the third half-hour; and then hold it at that point for twenty minutes.

If there is a considerable quantity of sputum, take about 1 c.c. from the bottom of the container, from the purulent or mucopurulent part of the specimen and place it in a sterile petri dish. Add from 1 to 2 c.c. of 5 per cent oxalic acid, depending upon the consistency of the sputum, thick, mucopurulent or purulent specimens requiring the larger quantity. Macerate thoroughly with a small, sterile wooden applicator. Pour into a sterile centrifuge tube and place in the incubator at 37.5° C. for one-half to three-quarters of an hour, shaking occasionally.

On removing the specimen from the incubator, add sterile normal salt solution until the tube is nearly full; mix, centrifugate and pour off the supernatant fluid except about 2 c.c. which is mixed thoroughly with the sediment. A slide is then made from the sediment mixture for the usual staining and microscopic examination. If no tubercle bacilli are found in this stained specimen, the material remaining in the centrifuge tube is seeded upon four tubes of medium, either by means of a sterile pipette, or, if the material is very thick, by a flamed wire loop.

After being incubated for about ten days, the cultures are carefully examined and, if the surface appears suggestive of a growth, a slight scraping is made for a slide which is stained by the usual methods. In this way a diagnosis often is possible before the appearance of definite colonies, but it must be remembered that such scraping with the wire loop may remove the only growth on the surface of the slant and prevent later confirmation of the diagnosis.

From all the known positive specimens of sputum seeded upon this medium, growth has appeared in from seven to eighteen days.

**AMYLOIDOSIS, Renal, in Relation to Renal Insufficiency, Dixon, H. M. Am. J. M. Sc. 187: 401, 1934.**

In 9,613 consecutive autopsies, 100 cases of renal amyloidosis were found.

Tuberculosis of the lungs (70 per cent) and bones (8 per cent) was the etiologic disease in 78 per cent of the cases.

The highest incidence of renal amyloidosis occurred in the third decade of life.

Twelve of the 46 cases in which sufficient evidence was available were associated with renal insufficiency.

Obstruction of the glomerular capillaries by amyloid deposits is a factor in the causation of renal insufficiency.

Hypertension is relatively infrequent in amyloid disease of the kidney. Of 35 cases with blood pressure readings, the systolic pressure was above 150 mm. in only 4 (12 per cent).

In the cases of renal amyloidosis with renal insufficiency, the kidneys were either normal in size or somewhat enlarged.

Renal amyloidosis may occasionally be associated with independent arteriolar nephrosclerosis.

Albuminuria is a fairly constant finding in renal amyloidosis.

**BLOOD; During the First Year of Life: The Anemia of Prematurity, Merritt, K. K., and Davidson, L. T. Am. J. Dis. Child. 47: 261, 1934.**

A study was made of groups of untreated premature and immature infants as to the behavior of the erythrocytes, hemoglobin, reticulocytes and platelets throughout the first year, and as to neonatal bleeding time and coagulation time. The values at birth and the lowest levels of the erythrocytes, the hemoglobin and the reticulocytes; the value at birth and the highest level of the platelets, and the mean values for all of these elements in the blood, after the blood had reached an equilibrium for the period studied are reported. The values for the bleeding and the coagulation time at birth are also reported.

A study was made of premature and immature infants who received iron and liver and iron, with respect to their erythrocytes, hemoglobin, reticulocytes, and platelets.

Premature infants treated with iron or liver and iron after they became anemic showed a greater decline of erythrocytes and hemoglobin and responded more slowly to treatment than did those who received antianemic therapy from birth.

The level of the reticulocytes from month to month in the combined groups of untreated premature and immature infants showed a negligible difference as compared with the level of the combined groups of treated infants.

The platelet values for the treated premature and immature infants throughout the first year are reported. No influence of antianemic therapy on the platelets has been found.

This study reiterates the fact that in the majority of premature infants a more or less severe anemia develops. An anemia of milder degree develops in immature infants. This anemia cannot be entirely prevented, but it can be ameliorated by the early administration of iron in large doses. Iron in the form of iron and ammonium citrate (50 per cent aqueous solution) has proved to be a satisfactory preparation in the amelioration and the treatment of the anemia. A dosage of 0.3 gm. (0.05 gm. of reduced iron) per kilogram appears to be adequate.

**TUBERCULOSIS, The Corrected Sedimentation Rate (C.S.R.) In, Freedman, S. Am. Rev. Tuberc. 29: 198, 1934.**

The corrected sedimentation rate (C.S.R.), arrived at by the Rourke and Ernstene method (J. Clin. Invest. 8: 545, 1930) gives the most accurate data in the study of blood sedimentation rates and should be the method of choice in further investigations.

Approximately 250 cases of pulmonary tuberculosis are analyzed from the standpoint of the C.S.R.

The grouping of patients according to their C.S.R. agrees well with their clinical classification.

Tuberculous activity, as measured by both the C.S.R. and the clinical classification, and positive sputum are more frequently found together than are activity and negative sputum.

Patients without rales have less activity, as measured by both the C.S.R. and the clinical classification, than patients with rales.

Patients without fever may or may not have an elevated C.S.R. depending on the amount of latent activity. Patients with fever almost invariably have an elevated C.S.R.

There is no correlation between the duration of the disease of the patients in this sanatorium and the C.S.R.

Gain in weight may or may not be associated with a normal C.S.R., depending upon the amount of latent activity. Loss of weight is almost always associated with an elevated C.S.R.

Patients with complications show the highest C.S.R.

Patients receiving artificial pneumothorax therapy show a somewhat lower average C.S.R. than do the other patients. Female patients receiving artificial pneumothorax show a distinctly greater percentage of normal C.S.R.

Patients with thoracoplasty have a definitely lower average C.S.R.

The C.S.R. proved to be of aid in the differential diagnosis of early and doubtful cases, being normal in all those cases that were discharged as nontuberculous.

Haematocrit values were higher in male patients than in female patients. Patients with low haematocrits had a much greater percentage of elevated C.S.R. than those with normal haematocrits.

A more widespread use, in sanatoria, of the sedimentation test reported by means of the C.S.R. is urged.

#### **TUBERCULOSIS: Four Years' Experience With Examinations of Material Obtained by Gastric Lavage, Lester, V. Am. J. Dis. Child. 47: 322, 1934.**

Using the method described below it has been shown that children under ten years of age secrete by no means as many tubercle bacilli as older children and adults. It must be remembered, however, that whereas samples were secured from all children with disease or suspected disease, a certain selection was made by the hospitals as to the adults, as no material obtained from gastric lavage was sent in from patients with microscopically positive expectoration. Thus a large number of the most infectious cases escaped tabulation, and there is no doubt that the difference in bacteriologic infectivity between the two age groups is still greater than appears from the tables. It remains for the clinicians to decide how great a danger these children present to persons in their environment. However, as no means are yet available for pointing out the relatively few small children who intermittently secrete many tubercle bacilli, theoretically, it will be best to keep children with positive gastric contents separate from persons who presumably are especially sensitive to infection, i.e., small children, particularly those who are ill.

*Technic.*—From 200 to 300 c.c. of the material obtained by gastric lavage is allowed to stand from eighteen to twenty-four hours, after which the sediment is sent to the laboratory. It is centrifugated, and smears are made for microscopic examination. The remainder is divided into two portions, one of which is homogenized with 4 per cent sodium hydroxide; the other, with 6 per cent of sulphuric acid by volume.

The soda homogenization proceeds at 37° C. for about twenty minutes, the glass being shaken now and then. After centrifugation at high speed for twenty minutes the supernatant fluid is removed, and the sediment is neutralized with 2 drops of 8 per cent hydrochloric acid; half of this is seeded with a Pasteur pipette in three Lowenstein tubes. The rest, together with some of the acid-homogenized material, is inoculated, partly subcutaneously and partly intraperitoneally, into a guinea pig. For the acid homogenization about 5 c.c. of 6 per cent sulphuric acid is added, and the sample after vigorous shaking

is allowed to stand in darkness for five minutes at room temperature. After the addition of from 10 to 15 c.c. of saline solution and brief centrifugation, the rather voluminous sediment is seeded in three tubes with a platinum loop. The rest is mixed with the part of the soda-homogenized portion that is intended for the inoculation of guinea pigs. In all cases in which there is sufficient sediment to permit of division, this technic is now used as a matter of routine, as it has been found that some strains best withstand the acid homogenization, whereas others give better results with soda homogenization. Thus parallel utilization of both methods should offer the best chance of obtaining positive results.

The tubes are sealed with paraffin, incubated at 38° C. and examined once a week. As soon as a culture is found to be positive, generally after from eighteen to twenty-five days, a smear is made for microscopic confirmation. The one who has sent the specimen is then notified to this effect, and the culture is put aside for subsequent counting of colonies and perhaps determination of type. If there is no growth after six weeks, the results of cultivation are considered negative, and the doctor who has sent the culture notified. The tubes are observed, however, for another four weeks, as a few colonies grow as late as this.

**SYPHILIS**, A Study of Jaundice In, Wile, U. J., and Sams, W. M. *Am. J. M. Sc.* 187: 297, 1934.

Jaundice following the use of arsenobenzenes in the treatment of syphilis is a not infrequent complication, and its relative incidence in our series and in those reported by others indicates that a high degree of hepatotoxicity is exerted by the drugs.

The low incidence of jaundice in untreated syphilis, 0.18 per cent, as compared with a percentage of 1.37 following arsphenamine, further incriminates these drugs as factors.

It is not believed that posttherapeutic jaundice can be regarded as a hepatocurrence, as is stated by Milian.

The symptomatology and the physical findings of the late or delayed type of posttherapeutic jaundice so closely approach those of infectious or so-called catarrhal jaundice as to make differential diagnosis possible only upon the history. The blood findings are not constant or characteristic.

A possible relation of postarsphenamine jaundice to the incidence of infectious jaundice, as pointed out by Stokes, Ruedemann and Lemon, is to some extent borne out by our studies.

About 60 per cent of the cases of posttherapeutic jaundice occurred in a period of three years, during which time almost half of the cases of infectious jaundice studied for comparison also occurred.

Extraneous associated factors exerting hepatotoxicity, such as pregnancy, malaria and alcoholism, play a minor rôle.

The pathologic diagnosis of acute yellow atrophy in 2 fatal cases studied, as well as others reported in the literature, indicate that the icterus results from a severe intoxication and destruction of the liver substance analogous to other forms of poisoning leading to acute yellow atrophy.

Until more accurate means are at hand to determine susceptibility and liver function, and until the drug is modified to make it less hepatotoxic, postarsphenamine jaundice will continue to be among the severe complications of the modern treatment of syphilis.

**STREPTOCOCCI**, Study of the Physiological Properties, Physical Properties, and Virulence of a Group of, Thompson, R. L., and Megrall, E. *Am. J. Hyg.* 19: 457, 1934.

In a group of 120 streptococcus strains, chiefly of human origin, a high correspondence was found between plate hemolysis, test tube hemolysis and the limiting hydrogen ion concentration.

As a rule, the same streptococcus strains were inhibited by sodium chloride, sodium ricinoleate, ox bile and by certain dyes. Growth of most of the beta and about half of the nonbeta strains was uniformly inhibited by these agents.

Streptococcus strains which were resistant to the bacteriostatic reagents possessed low isoelectric points. Strains which were nonresistant had high isoelectric points.

All strains virulent for white mice produced beta hemolysis. Likewise, of the strains tested, all cultures found to be toxigenic were of the beta type.

Individually or collectively, the above tests failed to separate this group of streptococci into smaller groups of significant meaning.

**FECAL BACTERIA:** Differentiation of *A. Aerogenes* and *A. Cloacae* on the Basis of the Hydrolysis of Sodium Hippurate, Hajna, A. A., and Damon, S. R. *Am. J. Hyg.* 19: 545, 1934.

The hydrolysis of sodium hippurate by *A. aerogenes* may be used as a further reliable cultural means of differentiating this species from *A. cloacae* in addition to, or in place of, the usual gelatin liquefaction test or reaction in adenite and inosite.

In determining hydrolysis of sodium hippurate the ferric chloride test may be carried out but the result may be anticipated by simple inspection as the cultures of *A. cloacae* remain clear in contrast to the turbidity seen in the cultures of *A. aerogenes*.

**BLOOD, Studies of, in Normal Pregnancy,** Dieckmann, W. J., and Wegner, C. R. *Arch. Int. Med.* 53: 345, 1934.

The normal figures for serum protein and fibrin reported in the literature for both nonpregnant and pregnant women are based on a relatively small number of determinations, and the data on the latter are especially confusing, either because of the methods used or because serial determinations on the same women were not made.

Determinations of serum protein, fibrin and plasma volume were made on various patients for the different periods of pregnancy and the puerperium. The results were studied statistically, and the following conclusions were made:

1. The changes in percentage of serum protein and in the amount of serum protein per kilogram of body weight are of no significance.

2. The changes in percentage of fibrin and in amount of fibrin per kilogram of body weight are significant. At term the former has increased 20 per cent and the latter 30 per cent.

A similar study in which the observations were made on the same women indicates that:

1. The serum proteins at term are from 0.3 to 15 per cent, average 7 per cent, below those of the first trimester. There is a still further decrease the first few days postpartum and then a slow rise, reaching a normal figure at about eight weeks postpartum.

2. The amount of serum protein per kilogram remains rather constant during pregnancy, ranging from 2.5 to 3.5 gm., with an average that is approximately 3.0 gm. per kilogram.

3. Determinations of the total amount of serum protein indicate that there is an average increase of 14 per cent at from twenty-six to thirty-five weeks, and an increase of 18 per cent at term. The figures postpartum are extremely variable, with the major portion of the patients showing a decrease which, after the first week, is almost negligible.

4. Figures for the percentage of fibrin show marked variations, but there is an average increase at term of 10 per cent. At eight weeks postpartum the figures are still above normal.

5. The grams of fibrin per kilogram show a more marked increase during pregnancy, reaching a maximum during the first week postpartum and then slowly decreasing, but at eight weeks the figures are still above normal.

6. Determinations of the total amount of fibrin indicate an average increase of 23 per cent at from twenty-six to thirty-five weeks, and an increase of 40 per cent at term. During the first week postpartum there is a slight decrease amounting to 5 per cent, but at eight weeks the average decrease is 26 per cent, thus furnishing additional evidence that there had been an increase in pregnancy.

## ABSTRACTS

7. The decrease in serum protein, even if the loss were all albumin, is insufficient in itself to cause physiologic edema. Changes in the osmotic pressure, surface tension and base-binding power of the proteins out of proportion to the decrease seem to indicate that there are intrinsic alterations in the proteins themselves.

**TRYPANOSOMES, A Simple Method for Isolating Single, Topacio, T. Philippine J. Sc.**  
51: 631, 1933.

The method consists essentially in making a stock suspension of trypanosomes in 30 per cent plasma of such strength that when a small drop is examined under the low power of the microscope, it is found to contain 3 or 4 organisms. From this stock suspension a series of minute drops are transferred by means of a capillary glass rod with a rounded end to a strip of sterile cellophane paper mounted on a glass slide by means of sterile serum. About 10 separate drops are deposited on the cellophane paper 8 mm. apart. The drops are then examined under the low power of the microscope. Immediately a drop is recognized to contain only a single organism, a little more 30 per cent plasma is superimposed, and the observer then passes to the examination of the other drops. Each drop with a single organism is removed from the slide by cutting a small square through the cellophane paper mount around the drop.

**OCCULT BLOOD, An Improved Test for Occult Blood, Especially in the Urine, Stone,**  
W. J., and Burke, G. T. J. A. M. A. 102: 1549, 1934.

1. Orthotolidine 1 per cent in chemically pure methyl alcohol. (It dissolves with slight difficulty and keeps at least ten months.)
2. Glacial acetic acid one part and commercial hydrogen peroxide two parts. (This keeps for three or four months, probably longer.)
3. Fifteen cubic centimeters of urine is centrifugated at about 1,500 revolutions per minute for five minutes. The supernatant fluid is poured off. A portion of the sediment is prepared for microscopic examination in the usual way. To the remaining sediment two drops of the orthotolidine solution is added plus two or three drops of the acid-peroxide solution. In the presence of blood cells aggregating 100 per c.mm. of sediment (approximately 1,350 per c.c. of urine) a greenish blue color develops, lasting about one minute. In the presence of from 300 to 500 red cells per c.mm. of sediment (approximately 4,000 to 6,500 cells per c.c. of urine) a deeper blue color develops lasting about one minute. In the presence of larger numbers of red cells, aggregating 1,000 per c.mm. of sediment (approximately 13,000 per c.c. of urine) as in hemorrhagic Bright's disease (glomerulonephritis) a deep blue color develops lasting two minutes or longer.

Undiluted blood serum, 10 per cent sodium hydroxide, strong trisodium phosphate solutions and probably other strong alkalis will give positive reactions. Pus cells or any of the common organic or inorganic constituents found in the urine do not give positive reactions.

**SCHICK TEST, Comparison of Dick Test and, in Mothers and Newborn Infants, Rothholz, A., and Kuttner, A. G. Am. J. Dis. Child. 47: 559, 1934.**

Forty-six children who had given positive reactions to the Schick test before tonsillectomy were retested about six months after the operation. Eighteen per cent of them gave negative reactions.

Of the 47 nontonsillectomized controls who gave positive reactions to the Schick test, 21 per cent gave negative reactions after about six months.

All the subjects came from congested urban districts and were mostly Porto Rican immigrants.

The carrier rate was not higher in the controls than in the tonsillectomized children.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

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### Histology\*

THE influence of the work of Ramou-Cajal has been so marked and extensive that this volume will be received with great interest.

While primarily a textbook for the student, it will be without doubt a welcome work of reference. The final chapter on histologic methods and the appendix on the preparation of different tissues contain a wealth of information for the laboratory worker.

The volume can be recommended without reserve.

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### Benign, Encapsulated Tumors in the Lateral Ventricles of the Brain: Diagnosis and Treatment†

THIS volume, which summarizes the results of fifteen years of observation and practical experience, presents clearly the advances which have been made in the surgical attack upon tumors in what, for many years, was regarded as largely forbidden territory, the brain.

Few men have been more closely associated with or are better fitted to discuss this subject than Dr. Dandy. His book may be accepted as a record of extensive experience carefully and impartially evaluated. Its scope is indicated by the table of contents: Small Primary Tumors of the Lateral Ventricles; Invasive and Malignant Tumors of the Lateral Ventricles; the Larger Encapsulated Tumors Causing Symptoms; Analysis of Signs and Symptoms in All Cases (this series and the literature); Localization of Tumors in the Lateral Ventricles by Ventriculography; Operative Treatment of Ventricular Tumors; Pathology. An extensive bibliography is attached supplemented, in the text, by two extensive tables.

A series of 15 cases is reported in detail with three deaths.

The clinical signs and symptoms are analyzed in detail and ventriculography emphasized as an accurate means of diagnosis and localization, this procedure when correctly used being without risk to life or function.

The susceptibility of these tumors to surgical removal is clearly discussed and described.

This book may be recommended without reserve as a valuable contribution to this subject.

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### The South African Institute for Medical Research‡

HOW many of us even think of South Africa in connection with medical research and yet here is a large institution with excellent buildings, good equipment and a staff consisting of 69 Englishmen, 2 Asiatics, and 37 African natives and an expenditure of \$280,000.00 in 1933.

The Department of Bacteriology reports work on plague, pneumonia, rabies and a disease of their native *Namagua gerbilles* somewhat resembling plague and caused by *Pasteurella*

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\*Histology. By S. Ramon-Cajal, revised by J. F. Tello-Munoz. Authorized translation from the tenth Spanish edition by N. Fernan-Nunez. Cloth, pp. 738, 535 figures. William Wood and Co., Baltimore, Md.

†Benign, Encapsulated Tumors in the Lateral Ventricles of the Brain; Diagnosis and Treatment. By Walter E. Dandy, M.D., Adjunct Professor of Surgery, Johns Hopkins University. Cloth, 189 pages, 83 figures, Williams & Wilkins Co., Baltimore, Md.

‡The South African Institute for Medical Research. Annual report, 1933. Johannesburg.



*desmodilli*. In the Department of Industrial Hygiene their most pressing problem is silicosis; in biochemistry, the native diet. In pathology the chief work seems to have been on cancer and in entomology, the vectors of malaria. There is also a routine division which makes numerous examinations (in 1933, 97,670) which may be public health, clinical pathologic, parasitologic, or medicolegal.

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### Ueber die Sogenante Primäre Kryptogenetische oder Metastatische Streptokokkenperitonitis\*

THIS is a thesis which was defended by the author before the medical faculty of the University of Helsingfors April 1, 1933.

From a study of the literature (100 references) and the 67 cases which he reports he concludes that primary streptococcal peritonitis is a metastatic manifestation of a general sepsis. Usually it is the first metastasis and because of its high mortality usually the last.

When the course of the attack is stormy from the start he finds that an operation is not worth while; his mortality in the unoperated cases being 100 per cent and in the operated cases, 94 per cent. In milder cases an operation may be indicated.

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### Handbuch der Chemotherapie†

THIS is Part II of the comprehensive German *Handbook of Chemotherapy* the first part of which was reviewed in the June number of this Journal. Part II takes up the metallic compounds, metal by metal, in a characteristically German way. It starts off with the arsenic compounds, gives first a general introduction and history of their use and then divides them into the acyclic compounds, the phenyl arsenic acid compounds and the arsenobenzene compounds. Under each head the individual drugs are discussed. Take, for example, atoxyl. He first gives its chemistry including a graphic formula, then its pharmacology and toxicology and then the therapeutic results. In this way 207 pages are given to arsenic including 2,900 references to the literature. In a similar way iodine is given 58 pages with 1,600 references, mercury 75 pages with 2,000 references, etc. The eight major metals so treated are arsenic, antimony, bismuth, iodine, copper, silver, gold and mercury; eleven other metals are given minor consideration.

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\*Ueber die sogenante primäre kryptogenetische oder metastatische streptokokkenperitonitis. (Concerning the so-called primary cryptogenetic or metastatic streptococcal peritonitis.) By U. Nordlund, Helsingfors, 1933.

†Handbuch der Chemotherapie. Zweiter Teil. Metallderivate, von Dr. Viktor Fischl und Prof. Dr. Hans Schlossberger. Fischers Medizinische Buchhandlung, Leipzig, 1934.

# The Journal of Laboratory and Clinical Medicine

VOL. 20

FEBRUARY, 1935

No. 5

Editor: WARREN T. VAUGHAN, M.D.  
Richmond, Va.

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## EDITORIAL

### Inversion of the Uterus

IN EVERY field of medical practice one finds at least a few conditions which are so unusual or so infrequent as to merit the interest of physicians in general. Such a condition is inversion of the uterus, occurring in an estimated frequency of 1 in every 250,000 deliveries.

Uterine inversion usually occurs postpartum and appears often to follow too violent traction on the cord or manipulation of the fundus through the abdominal wall in Credé technic. At the same time a surprisingly large proportion occurs in the absence of either of these procedures and appears to be spontaneous. The first serious discussion of this condition was by John Crosse of England in 1846. There have been numerous reports in the literature since then but, even so, this complication of pregnancy appears to be very rare indeed. The generally accepted explanation is that during postpartum contractions of the fundus, the thin rather weakened uterine wall at the previous placental

site invaginates spontaneously. The thicker surrounding wall then grasps this invaginated portion and gradually milks it down through the cervix and out into the vagina. This may occur immediately after delivery or after hours or days. "After any portion of the uterus becomes indented to a considerable extent the rest of the organ seizes this invaginated portion as it would grasp a foreign body, and, in attempting to expel it, turns itself inside out." Undoubtedly there are many mild unrecognized cases. Spontaneous readjustment of a partial inversion probably not infrequently takes place and is seldom recognized. This matter of downward traction on the incupping fundus, by the rest of the uterus, probably explains the many cases of spontaneous inversion. When we think of the brutal force that is sometimes applied in attempts to expel the placenta, without resulting inversion, it becomes obvious that there must be some other spontaneous factor at work in a large proportion of cases. Findley estimates that one-third of all puerperal inversions arise spontaneously, in the absence of traction on the cord or pressure from above. Thorne found 54 per cent of 437 cases to be spontaneous.

Phaneuf sums up the etiology as follows: "Although spontaneous inversion may occur, one of three factors is usually necessary for the production of this condition; namely, undue relaxation of the uterine wall, pressure from above, and traction on the fundus from below. In addition the employment of the Credé expression on a relaxed uterus, or before placental separation, the erect posture in labor, a short cord, straining of the abdominal muscles during labor, coughing, sneezing, fundal implantation of the placenta, and the pressure of submucous fibroids during pregnancy are all contributory factors."

Reeve states, "the action may occur independently of anything done or omitted." Cases have been ascribed to coughing and straining at stool though in such cases it is probable that there had been a preexisting unrecognized partial inversion of the fundus.

Smither and Holloway estimate that the complication occurs once in two or three hundred thousand cases. They state that in St. Petersburg there was no case recorded in over 250,000 deliveries. In Dublin there was one case in 200,000. Few even among those who are specializing in obstetrics have seen a case. Phaneuf found in a review of the literature, one case in 125,000 labors.

This always becomes a serious complication of pregnancy if the inverted fundus passes the cervix. The symptoms are shock and hemorrhage. The death rate is high, being variously estimated as from 14 per cent to over 75 per cent. There is some difference of opinion regarding treatment. Most authors recommend replacement as soon as the complication has been recognized. Sometimes manual replacement is possible, at others laparotomy is necessary. In the presence of severe shock most authors recommend transfusion and supportive therapy. Others feel that the danger from delay requires replacement even in the presence of shock.

The condition is so infrequent that it is often unrecognized, even by good men. Foster Kellogg writes, "Williams says the diagnosis is easy, and we agree that it should be, but in our experience we have seen it overlooked by well-trained obstetricians." D'Errio, in his review of the cases observed at the

Boston City Hospital, and of the importance of prompt recognition, stresses his feeling that an effort should be made to emphasize the importance of this complication in medical schools, and in the profession at large.

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—W. T. V.

## NEWS AND NOTES

### The American Neisserian Medical Society

The American Neisserian Medical Society was founded on June 12, 1934. It is dedicated to the promotion of knowledge in all that relates to the gonococcus and gonococcal infectious, that there may be attained improvement in the management of gonorrhea and a reduction in its prevalence. There are 115 charter members and the officers are:

Dr. Edward L. Keyes, New York, Honorary President	}	Executive Committee
Dr. J. Dellinger Barney, Boston, President		
Dr. P. S. Pelouze, Philadelphia, Vice-President		
Dr. A. L. Clark, Oklahoma City		
Dr. Walter Clarke, New York		
Dr. R. D. Herrold, Chicago		
Dr. N. A. Nelson, Boston	}	
Dr. Oscar F. Cox, Jr., Boston, Secretary-Treasurer		

The Society plans to carry out the following program:

- The scrutiny of the management of gonorrhea in both male and female.
- Clinical and laboratory research in the diagnosis, medical and social pathology, and the treatment of gonorrhea.
- Dissemination among the medical profession and the public of authoritative information concerning gonorrhea.

Membership is limited to:

- Residents of the United States or its territories, Canada or Mexico.
- Graduates of a medical school recognized by the American Medical Association.
- Those who are engaged in some phase of the management of gonorrhea.

Invitation to membership is extended to all qualified physicians who desire to work for improvement in the management of gonorrhea. Application blanks can be obtained from the undersigned.

OSCAR F. COX, JR., M.D., Secretary  
 475 Commonwealth Ave.  
 Boston, Mass.

# *The Journal of Laboratory and Clinical Medicine*

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VOL. 20

MARCH, 1935

No. 6

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## *CLINICAL AND EXPERIMENTAL*

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### THE VOLUME THICKNESS INDEX OF THE ERYTHROCYTE OF MAN\*

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RUSSELL L. HADEN, M.D., CLEVELAND, OHIO

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NORMALLY, the erythrocyte of man is a biconcave disc with a remarkable constancy in shape and relationship of diameter to thickness. With a variation in volume of the cell, the changes in diameter and thickness are usually relatively equal. Thus, in the characteristic macrocytosis of pernicious anemia, both the diameter and thickness are increased, and in microcytic anemia the two dimensions are equally decreased. In certain clinical conditions, however, the normal relation between diameter and thickness is altered. With obstructive jaundice, the diameter increases at the expense of the thickness, so that the cell becomes flattened or less globular; in congenital hemolytic jaundice, the reverse takes place and the cell becomes more globular. The importance of this fact in the diagnosis of congenital hemolytic jaundice and of the relation of the shape of the erythrocyte to its resistance to hypotonic sodium chloride solutions has been discussed elsewhere.<sup>1</sup>

There is no satisfactory method for direct determination of the thickness of an erythrocyte. Emmous<sup>2</sup> measured the thickness of rouleaux of red cells in wet preparations and calculated the thickness after counting the number of cells in each rouleau measured. The thickness can be calculated, however, from the diameter and corpuscular volume. Von Boros<sup>3</sup> has prepared a three dimensional chart (Fig. 1) by which the thickness of an erythrocyte can be determined directly from the diameter and corpuscular volume. To calculate the thickness, the intersection of the diagonal line, representing the measured

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\*From the Cleveland Clinic.

Received for publication, July 24, 1934.

mean diameter, with the vertical line, representing the measured mean corpuscular volume, is spotted. A line down from this point crosses the graduated vertical line at the left at the mean erythrocyte thickness.

The thickness of the erythrocyte is important chiefly because it allows exact determination of the shape of the cell, if the volume and thickness are known. Von Boros<sup>4</sup> suggested a "thickness index" to express numerically the relative thickness. He determined the "thickness index" by dividing the mean corpuscular volume as determined directly from the cell count and hematocrit reading by the mean corpuscular volume, as calculated from the mean cell diameter. The calculation of the mean corpuscular volume from the mean cell diameter is made by a rather complicated formula, evolved by von

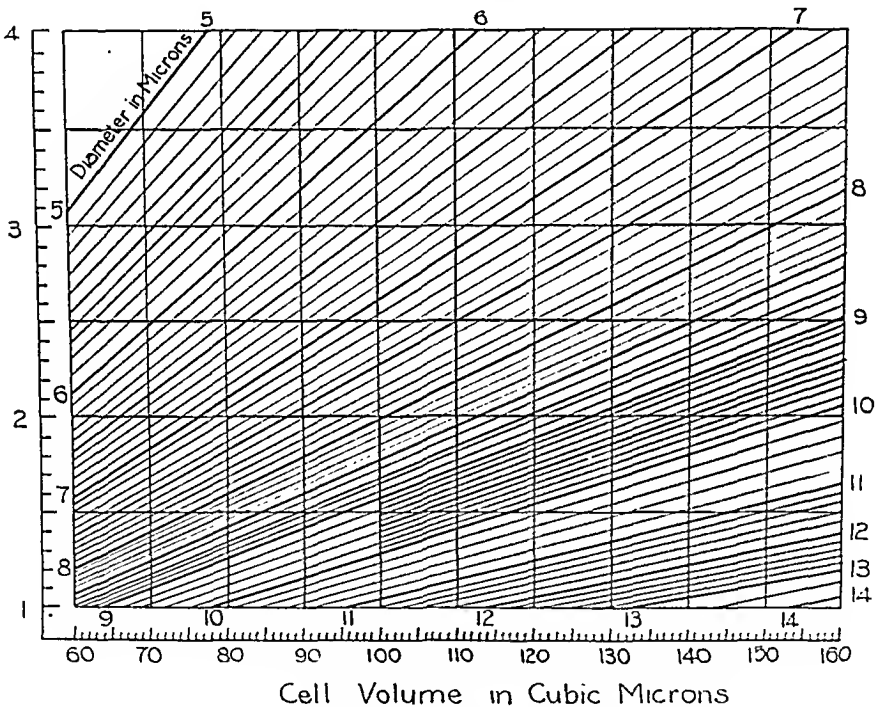


Fig. 1.—Three dimensional chart for calculating the thickness of the erythrocyte from the diameter and volume (after von Boros<sup>4</sup>).

Boros and applicable only when a variation from normal in the diameter is accompanied by a similar and equal variation in thickness.

A true "thickness index" should indicate only the thickness relative to normal. The index suggested by von Boros takes into consideration both volume and thickness, and so expresses numerically the tendency to globular form. In pernicious anemia, the thickness is increased with the macrocytosis but the diameter is equally increased. A true "thickness index" would thus be greater than 1.00. Since the change in all dimensions is equal, however, the relative shape or globularity of the cell is unchanged. The index proposed by von Boros is accordingly best termed the volume thickness index, since it shows the relation of volume and thickness relative to normal. The volume thickness index is defined as the relation between the measured mean

corpuscular volume and the calculated corpuscular volume of a cell corresponding to the measured mean diameter and having the normal relation between diameter and thickness. If the volume thickness index is greater than 1.00, the cell is thicker than normal in relation to diameter and volume, and so is more globular than normal; if the index is less than 1.00, the cell is thinner than normal in relation to diameter and volume and thus is less globular than normal. The absolute thickness of a cell is best expressed in relation to the absolute diameter as the diameter thickness ratio.

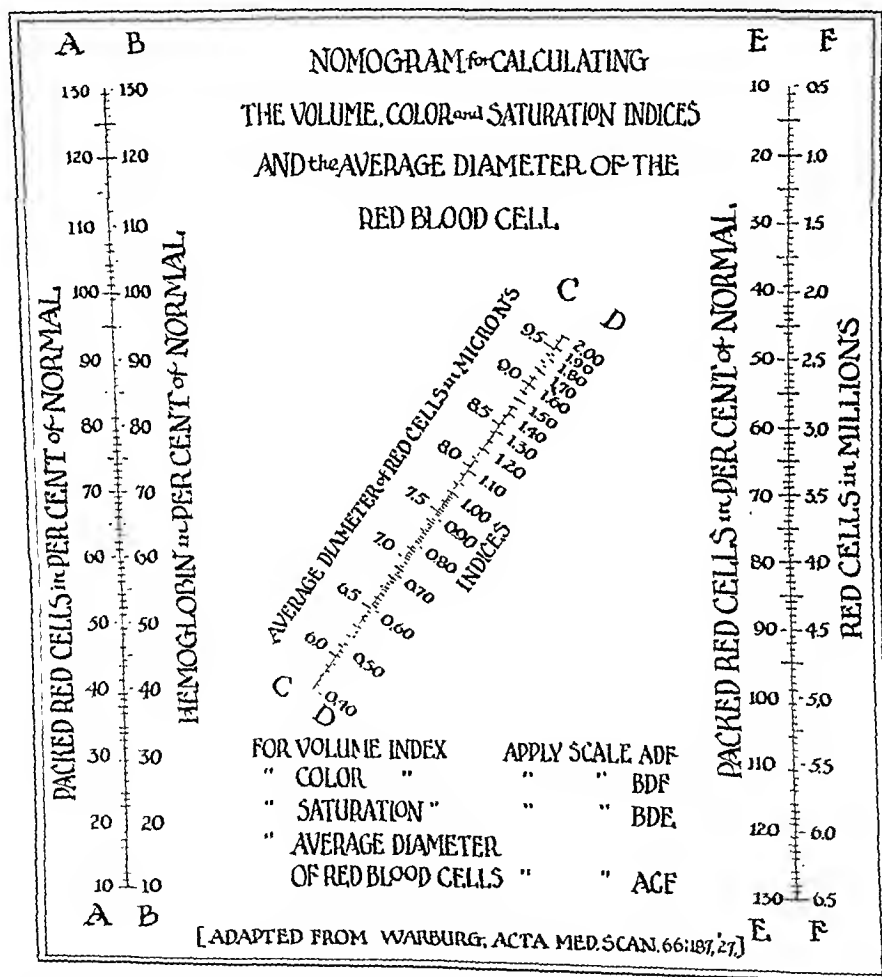


Fig. 2.—Nomogram for calculating the indices of the erythrocyte from the number, the hemoglobin and the hematocrit value of the erythrocyte. The mean diameter can also be calculated from the red cell count and hematocrit reading (after Warburg).

The volume thickness index can be calculated much more simply than as described by von Boros if a nomogram (Fig. 2) showing the volume index corresponding to the cell diameter is employed. It is estimated as follows:

1. Determine the mean corpuscular volume in cubic microns and the volume index of the unknown blood.
2. Measure the diameter of at least 200 red cells and calculate the mean erythrocyte diameter of the unknown blood.

3. Column D in the nomogram shows the volume index of the erythrocyte corresponding to different diameters. Find the volume index corresponding to the measured mean erythrocyte diameter of the unknown blood.

4. The mean corpuscular volume corresponding to the volume index is calculated by multiplying the normal mean corpuscular volume by the volume index. Calculate the mean corpuscular volume of the unknown blood by multiplying the normal mean corpuscular volume by the volume index found corresponding to the measured mean diameter of the unknown blood.

5. Calculate the volume thickness index (V-T I) by dividing the mean corpuscular volume of the unknown blood, as determined directly from the cell count and hematocrit reading, by the calculated mean corpuscular volume corresponding to the measured mean erythrocyte diameter.

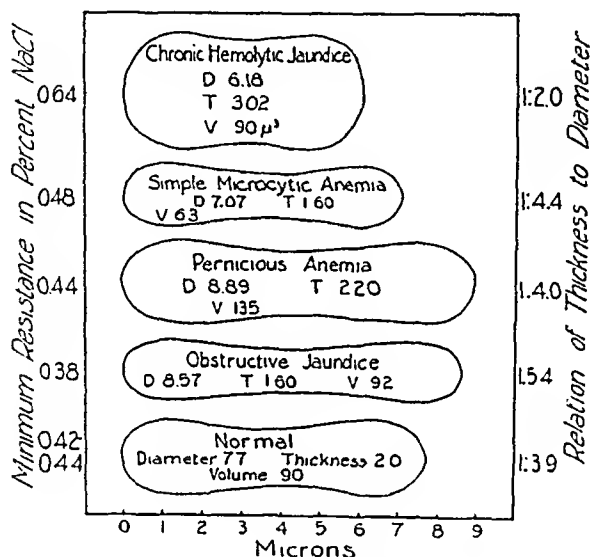


Fig. 3.—Cross-section diagrams of the mean erythrocyte in various clinical conditions.

#### ILLUSTRATIVE CALCULATION

Normal mean corpuscular volume	= 90	cubic microns
Normal erythrocyte diameter	= 7.7	microns
Normal erythrocyte thickness	= 1.95	microns
Normal diameter thickness ratio	= 7.7:1.95 or 4:1	
Normal volume thickness index	= 1.00	
Mean corpuscular volume of unknown blood	= 81	cubic microns
Mean erythrocyte diameter of unknown blood	= 6.5	microns
Mean erythrocyte thickness of unknown blood	= 2.45	microns
Diameter thickness ratio of unknown blood	= 6.5:2.45 or 2.6:1	

From the nomogram the volume index of a cell with a diameter of 6.5 microns (Column C) is 0.60 (Column D) if the thickness is decreased equally with the diameter. The corpuscular volume of this cell is  $0.60 \times 90 = 54$  cubic microns.

The volume thickness index (V-T I) =  $81/54 = 1.50$ .

#### SUMMARY

Knowledge of the exact measurements for all three dimensions of the mean erythrocyte may be of clinical value.

The thickness of a cell may be calculated if the diameter and volume are known.



The relation between the diameter and thickness is best expressed as the diameter thickness ratio.

The volume thickness index expresses numerically the tendency to globular form or the spherocytosis of the erythrocyte.

TABLE I  
TYPICAL CELL MEASUREMENTS

	MEAN ERYTHROCYTE			DIAMETER THICKNESS RATIO	ERYTHROCYTE VOLUME COR- RESPONDING TO MEASURED DIAMETER CUBIC MICRONS	VOLUME- THICKNESS INDEX
	VOLUME CUBIC MICRONS	DIAMETER MICRONS	THICKNESS MICRONS			
Normal	90	7.7	1.95	4:1	90	1.00
Pernicious anemia	135	8.89	2.20	4:1	139	0.96
Microcytic anemia	63	7.07	1.60	4.4:1	69	0.91
Obstructive jaundice	92	8.57	1.60	4:1	123	0.75
Chronic hemolytic jaundice	90	6.18	3.02	2:1	47	1.92

Methods for determining these measurements have been furnished and typical findings recorded. (Typical measurements in several clinical conditions are shown in Table I, and a cross-section view of cells corresponding to these measurements in Fig. 3.)

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# THE EFFECT OF THYMOL ON THE PROGRESS OF RABBIT MONILIASES\*

W. D. STOVALL, M.D., S. B. PESSIN, M.D., AND LOIS ALMON, PH.D.,  
MADISON, WIS.

## INTRODUCTION

THE proper treatment of pulmonary moniliases is, at present, an uncertainty. Iodine, iodides, gentian violet, and thymol have all been used with varying degrees of success. The need for laboratory experimentation is apparent. Thymol was the agent chosen for this study because it was the most active of any tried in preliminary test tube experiments.

The value of thymol as a bactericidal agent has long been known. More recently its fungicidal action has also been recognized. But in the treatment of disease the drug has been used primarily as an anthelmintic. Its use in other types of infection is still in the experimental stage.

A paper by Myers and Thienes, 1925, reports favorable results from the use of thymol in the treatment of fungous infections of the skin. In the treatment of two cases of pulmonary mycosis the results were contradictory. A later paper by Myers, 1927, gives data on the fungicidal properties of thymol and volatile oils in vitro. Thymol was the most active of any of the substances which he used. Mitchell in an address, 1927, expressed substantiation of the results of Myers and Thienes in the treatment of skin lesions. But aside from these reports the literature is devoid of information concerning the possible value of the drug in the treatment of mycoses.

With the standardization of morbid and lethal doses of *Monilia albicans* and *candida* for rabbits (Stovall and Pessin, 1933), it has become possible to measure the effects of thymol on the progress of infection. This is what has been attempted in the work herein reported. Studies on the fungicidal action of thymol in vitro and on some aspects of the pharmacology of thymol for rabbits were undertaken also in order to lay a more solid foundation for the investigation of prophylactic and therapeutic value.

## THE INHIBITORY AND CIDAL ACTION OF THYMOL ON MONILIA SPECIES

The value of a drug in the treatment of infectious processes lies in its ability to cause the death of the invading organisms, directly, by action on the organisms or, indirectly, by checking their multiplication and allowing the natural cidal agencies of the host to act upon them. The inhibitory or cidal action in laboratory media or aqueous suspensions, while not a measure

\*From the State Laboratory of Hygiene, University of Wisconsin.

Received for publication, July 20, 1934.

This work has been supported in part by a grant from the University Research Fund.

of the same reactions in an animal host, may nevertheless serve to indicate whether such reactions are at all possible.

#### CIDAL ACTION

The procedure to determine what concentrations of thymol would kill monilias and what period of time was necessary for the action was as follows.

Cultures were grown in 1 per cent glucose broth in centrifuge tubes for forty-eight hours, after which the growth was thrown down, the supernatant liquid removed and the appropriate strength of aqueous thymol solution added. Portions of the resulting suspension of organisms were removed at stated intervals to malt agar and glucose broth to test for viability. Six cultures were used throughout; two of *Monilia parapsilosis*, two of *M. albicans*, and two of *M. candida*. The thymol was allowed to act at 25° C.

One to one thousand,\* 1:2,000, and 1:5,000 dilutions of thymol were used for intervals of 1, 2, 5, 10, 15, 60, and 120 minutes with the following results: The 1:1,000 concentration killed five cultures in less than one minute, and all of the cultures in less than five minutes. The 1:2,000 concentration killed the two *M. candida* cultures in less than five minutes, the two *M. albicans* cultures in less than fifteen minutes, but failed to kill completely both of the cultures of *M. parapsilosis*.

In an attempt to determine the effect of the presence of NaCl in the reacting mixture of cells, thymol, and water, the thymol was dissolved in sterile 0.9 per cent NaCl solution, and the experiment continued in the manner just

TABLE I\*

GROWTH ON MALT AGAR OF MONILIA CULTURES SUBJECTED TO THE ACTION OF AQUEOUS THYMOL SOLUTION AND THYMOL DISSOLVED IN 0.9 PER CENT SODIUM CHLORIDE SOLUTION

CULTURE	CONCENTRATION OF THYMOL	TIME IN THYMOL SOLUTION					
		1 MINUTE		5 MINUTES		15 MINUTES	
		WITH NaCl	WITHOUT NaCl	WITH NaCl	WITHOUT NaCl	WITH NaCl	WITHOUT NaCl
P 3	1:1000	-	-	-	-	-	-
	1:2000	+	+	+	+	+	+
P 4	1:1000	-	-	-	-	-	-
	1:2000	+	+	+	+	+	+
A 7	1:1000	-	-	-	-	-	-
	1:2000	+	+	+	+	-	-
A 8	1:1000	-	+	-	-	-	-
	1:2000	+	+	+	+	-	+
C 3	1:1000	-	-	-	-	-	-
	1:2000	+	+	-	-	-	-
C 4	1:1000	-	-	-	-	-	-
	1:2000	+	+	-	-	-	-

\*Thymol action at 25°C. Incubation of malt agar at 37°C.

Streaks from the suspensions of organisms in salt solution without thymol showed growth throughout.

+ indicates growth.

- indicates no growth.

P indicates culture of *Monilia parapsilosis*.

A indicates culture of *M. albicans*.

C indicates culture of *M. candida*.

\*One gram of thymol will dissolve in one liter of water at 37°C., after three to four days. Since thymol is volatile, the resulting solution may not represent quite a 1:1,000 concentration, but will be designated as such for the purpose of comparison.

described, using 1:1,000 and 1:2,000 dilutions of thymol and removing portions of the suspension of organisms to malt agar at one-minute, five-minute, and fifteen-minute intervals. Controls consisted of suspensions of the organisms in thymol solution without salt and in salt solution without thymol. Table I gives readings from the malt agar streakings after four days. With only one culture, A8, was there any difference between the survival in thymol solution with salt and a similar solution lacking salt. It may be concluded, therefore, that the presence of NaCl has little effect upon the cidal power of thymol in aqueous solutions. The greater resistance of *Monilia parapsilosis* to the more dilute solution is of academic, if not practical, interest. In general, it would seem that thymol in concentration of 1:1,000 kills in less than one minute the three species of *Monilia* with which we deal.

#### INHIBITION

Cultures of *Monilia* are most commonly grown in this laboratory on malt agar or in 1 per cent glucose broth. These media are favorable for abundant growth. The addition of thymol to these media would, therefore, be a means of determining the inhibitory concentrations. Accordingly, amounts of the 1:1,000 aqueous solution were added sufficient to afford concentrations of 1:5,000 and 1:10,000 in the malt agar and glucose broth. A 10 per cent alcoholic solution was utilized in producing concentrations of 1:1,000 in the two media. The pH of the malt agar was 6.0; that of the glucose broth, 7.6. After inoculation, observations to detect growth were made from time to time over a period of two weeks. Brom-thymol-blue was added to the glucose broth to facilitate the detection of growth, since all of the cultures used readily produce acid (and gas) in this medium. From those tubes in which an acid reaction appeared, wet mounts were made for microscopic examination to rule out the possibility of a contaminating organism having been responsible for the production of the acid. Control tubes without thymol were also set up.

The results may be briefly stated. A concentration of thymol amounting to 1 part in 5,000 parts of malt agar inhibited growth for a period between forty-eight hours and four days; occasionally longer. In glucose broth, inhibition by this concentration was complete over a two-week period for 18 of the 19 cultures used in the experiment. The remaining one showed no growth at the end of the first week, but developed during the second week. Transfers from these tubes at the end of two weeks to glucose broth without thymol resulted in growth in every case, showing that the organisms had not been killed by the 1:5,000 concentration but merely prevented from growing.

It was desirable to see what effect the pH of the media might have upon the inhibitory power of thymol. For this purpose the malt agar was made up at a pH of 7.8 as well as 6.0, and the glucose broth at 6.2 and 7.8. With both media, inhibition was slightly aided by the alkaline reaction, but the difference was not striking. A thymol concentration of 1:4,000 was completely inhibitory for two weeks (the duration of the experiment) at both hydrogen ion concentrations.

Since inhibition or cidal effect in the presence of body fluids would be the test of the value of thymol in infectious processes, an experiment using thymol

in rabbit serum was also conducted. Due to the fact that a saturated aqueous solution of thymol contains only one part in a thousand, it was impracticable to prepare a thymolized serum having a greater concentration than one part of thymol to 5,000 of  $\frac{4}{5}$  serum, for the addition of more than one part in five of the aqueous solution would dilute the serum to such an extent as to destroy the value of the experiment. Hence the experiment consisted of tubes containing four parts of rabbit serum and one part of a saturated aqueous solution of thymol; with the same mixture lacking in thymol and with 1:5,000 thymol in glucose broth as controls. Three cultures, representing the three species of *Monilia* were used for inoculation. All of the tubes except the thymolized glucose broth tubes showed growth within twenty-four hours, indicating that a concentration of thymol of one part in 5,000 of serum does not inhibit multiplication of these organisms.

#### SOME ASPECTS OF THE PHARMACOLOGY OF THYMOL FOR RABBITS

The following work was undertaken to determine how large a dose of thymol rabbits can stand without injury, what proportion of it is absorbed from the alimentary tract, and how long it remains in the system. Relatively little work of this kind has been done with rabbits. Work by Livingston, 1921, indicates that these animals can endure a single dose of 2 grams of thymol for every kilogram of body weight; but he does not describe post-mortem findings; and there is no suggestion in his work as to what the effect of frequent, repeated doses might be. Seidell's work, 1915, gives data on absorption and retention for dogs and human beings. He found that practically the whole of the amount administered was absorbed, since only negligible amounts could be detected in the feces, and that that which was excreted in the urine passed off chiefly within twenty-four hours after the dose had been given. While it is probably safe to infer that rabbits would react similarly, we have nevertheless done work of the same kind with these animals.

The thymol was fed in olive oil solution through a rubber catheter acting as a stomach tube. Quantitative estimations of the amounts excreted in the urine were made from day to day by the following method: Sulphuric acid was added to the urine in the amount of 10 per cent by volume. The mixture was steam distilled, the distillate extracted with ether, the ether evaporated, and the residue, consisting almost entirely of thymol (confirmed qualitatively by Lustgarten's test\*) was weighed. The feces of two animals were similarly tested. After varying numbers and sizes of doses had been administered, the animals were killed and examined for gross and microscopic pathology.

It was desirable to determine the largest dose which could be tolerated repeatedly without ill effect, since upon the size of dose would depend the concentration in the blood and hence the efficacy of the drug.

After considering the figures in Livingston's paper (1921), we decided that a dose of one gram of thymol per kilo of body weight would be a sensible beginning. It was found, however, that while a dose of this order was not lethal it was deleterious to the well-being of the animal. The two rabbits fed

\*Fifty per cent KOH added. Mixture warmed. A few drops of chloroform added. Shaken. The appearance of a violet color indicates the presence of thymol.

with this amount of thymol lost in weight, excreted urine dark in color for a week after the dose had been given, and moped in their cages. One of them, T 2, also showed marked tissue changes at autopsy (Table II). This behavior led us to make more careful examinations of the urine of animals subsequently used in the experiments. Thymol recovery was made from only half of each sample; the amount recovered was multiplied by two to represent that which was present in the total sample. (The figures in Table II give the corrected

TABLE II  
THYMOL ADMINISTRATION TO NORMAL RABBITS

RABBIT	WT. IN GRAMS	THYMOL GIVEN GRAMS PER KILO BODY WT.	AMOUNT RECOVERED FROM URINE GRAMS PER KILO BODY WT.	AMOUNT RECOVERED FROM FECES GRAMS, TOTAL	TIME BETWEEN TIME OF LAST DOSE AND KILLING DAYS	PATHOLOGY
T 1	3220	1.0	0.266	0.028	4	345 g. loss in wt. No gross pathology
T 2	3750	1.0	0.222	0.020	16	Fatty changes in liver and kidney. Kidneys congested
T 4	2945	1.25	0.126	Not done	1	Albuminuria. Tissues like T 2 but to lesser degree
T 3	3800	0.5 0.5 0.4 0.5 0.4 Daily Intervals	0.135 0.122 0.037 0.159 0.096	Not done Not done Not done Not done Not done	Saved for further experiments	Urino normal
T 5	2555	0.4 0.4 0.3 0.4 0.4 Daily Intervals	0.028 0.041 0 0.088 0.040	Not done Not done Not done Not done Not done	Saved for further experiments	Albuminuria (due to pregnancy)
T 6	2865	0.4 0.4 0.4 0.4 0.4 0.4 0.4 2-Day Intervals	0.071 0.042 0.086 0.089 0.099 0.097 0.065	Not done Not done Not done Not done Not done Not done Not done	7	Urine: Albumin negative throughout; reducing sugar present; epithelial cells and leucocytes present in small number. Autopsy showed no abnormalities

values.) The other half of each sample was used for albumin and reducing sugar analyses and for microscopic examinations. Only two distillations of feces were performed because the amount excreted by this route was found to be negligible. The ether extract of the steam distillate of normal urine was also negligible in amount; hence the residue from ether evaporation of thymol urine is reported as pure thymol.

Doses of 0.5 gram per kilo of body weight were next investigated. The first rabbit (T 3) to receive such a dose was observed for a period of three days following the initial thymol feeding. During this time the rabbit ate and appeared otherwise normal. All of the thymol recovered from the urine was

found in the first twenty-four-hour sample. Slightly more than 25 per cent of the amount administered was thus obtained. The animal lost 50 g. during the three days, not a significant loss for so large an animal. Daily doses of the same size were then given for four days. During this time the rabbit ate normally, but the urine output was depressed, averaging 96 c.c. daily as opposed to an average of 203 c.c. before thymol dosage was begun. Also the color of the urine was darker during the period of thymol administration. Eight days after the last feeding the urine was again normal in quantity and appearance.

While the dose of 0.5 gram per kilogram of body weight was not markedly deleterious, the depressed amount of urine, abnormally dark in color, seemed evidence of some ill effect. The dose was therefore cut to 0.4 gm. per kilo. Rabbit T 5 received daily doses of this amount for five days. Rabbit T 6 received the same sized dose every other day for two weeks.

Rabbit T 5 showed consistent albuminuria, but this was probably due to pregnancy which was not discovered until later. The five young born seven days after the last dose of thymol were normal in size, but lived only two days, probably due to neglect on the part of the doe. The urine of rabbit T 6 gave no evidence of any pathologic condition. The blood nonprotein nitrogen of both rabbits remained within the limits of normal variation during the entire period of observation. Indications were that doses of 0.4 gram per kilo of body weight were not appreciably injurious.

It remained to determine whether there was a persistent uncomfortable irritation of the alimentary mucosa by the thymol. Rabbit T 4 was killed twenty-four hours after the administration of a large dose. There was no congestion or other abnormality of the mucosa or of the kidney. Later, rabbit T 3 was also given a large dose and was killed after four hours. This animal likewise showed no evidence of irritation.

Incidentally two random attempts were made to determine the concentration of thymol in the blood some time after giving the doses. In the first instance 40 c.c. of blood were drawn from the heart and steam distilled thirteen hours after the forced feeding of a dose aggregating 1 gm. per kilo of body weight. No detectable amount was recovered. In the second instance the rabbit was killed four hours after it had received a similar dose, and 100 c.c. were drawn from the heart. In this case the liver was removed, as well, and ground up with sand in a mortar, preparatory to steam distillation. The blood yielded 0.011 gm., indicating a concentration of approximately 1:10,000. The liver yielded 0.017 gm. That the product recovered from the steam distillation was thymol and not some other ether extractive was affirmed by the Lustgarten qualitative test.

Since the concentration in blood and tissues was so low even after the administration of a large dose, and since the thymol exists in the animal organism in compounds the efficacy of which for the destruction of *Monilia* is not known, the therapeutic value would seem by a priori reasoning to be dubious.

TABLE III  
EFFECT OF THYMOL IN RABBIT MONILLIASIS

RABBIT	DOSE OF ORGANISMS IN MILLIONS PER 100 G. OF BODY WT.	DOSE OF THYMOL IN GRAMS PER KILO OF BODY WT. ALSO FREQUENCY OF DOSAGE	LENGTH OF LIFE FOLLOWING INJECTION OF ORGANISMS	PATHOLOGY	
				GROSS	MICROSCOPIC
T 26	1.25	None	Killed 7 hours	Lung: Petechial hemorrhages. Bile: No monilia. Urine: Few monilia. Same as T 26.	Kidney: Few monilia; multiplication scarcely begun. No hyphae. Same as T 26.
T 27	1.25	0.45 First day	Killed 7 hours	Lungs: Occasional petechial hemorrhages. Kidneys: Occasional small cortical abscesses.	Kidney: Monilin and mycelium present. Liver: No cellular areas.
T 28	1.3	None	Killed 25 hours	Gallbladder empty. Urine culture pos. Kidneys: Negative. Bile cul. 2 colonies. Urine culture pos.	Same as T 28.
T 29	1.5	0.5 First day	Killed 24 hours	Generalized milinary monilliasis.	Lung: Edemat. Kidneys: Abscesses containing mycelium and conidia. Liver: Abscesses.
T 5	1.5	None	Died Third day	Kidney: Only occasional abscesses. Culture pos. Bile: Culture pos. Urine culture pos.	Kidneys: Healed areas present. Chronic nephritis. Liver: Slight fatty changes.
T 9	1.1	0.4 First day	Killed Twelfth day	Kidneys: Many abscesses. Bile: Conidia and hyphae present. Urine culture pos.	Kidneys: Abscesses contain conidia but no hyphae. Liver: A few Monilia.
T 10	0.5	None	Killed Sixth day		



TABLE III—Cont'd

RABBIT	DOSE OF ORGANISMS IN MILLIONS PER 100 G. OF BODY WT.	DOSE OF THYMOL IN GRAMS PER KILO OF BODY WT. ALSO FREQUENCY OF DOSAGE	LENGTH OF LIFE FOLLOWING INJECTION OF ORGANISMS	PATHOLOGY	
				GROSS	MICROSCOPIC
T 7	0.6	0.45 First day	Killed Sixth day	Kidneys: A few abscesses. Culture positive. Bile: Negative.	Kidneys: Monilia in abscesses so scarce as not to be detected in sections. Liver: Marked fatty changes. A few Monilia.
T 30	1.5	None	Killed Seventh day	Generalized military moniliasis.	
T 23	1.0	0.5 Daily	Killed Sixth day	Generalized military moniliasis.	
T 18	0.63	None	Killed Seventh day	Moderate number of lesions in kidneys.	
T 32	0.8	0.4 1 dose	Killed Seventh day	Moniliasis more extensive than in T 18.	
T 33	0.67	0.4 1 dose	Killed Seventh day	Moniliasis more extensive and decidedly more severe than T 18.	
T 22	1.6	None	Killed Eighth day	Generalized military moniliasis.	
T 31	1.6	0.5 Daily	Killed Eighth day	Kidneys: Moderate no. of military cortical abscesses. Bile: Culture pos.	
T 12	1.3	None	Killed Twelfth day	Kidneys: Many military abscesses. Bile: Culture pos. Urine: Culture neg.	Kidneys: Moderate granulomatous areas. Liver: Occasional budding; Monilia present.

TABLE III—CONT'D

RABBIT	DOSE OF ORGANISMS IN MILLIONS PER 100 G. OF BODY WT.	DOSE OF THYMOL IN GRAMS PER KILO OF BODY WT. ALSO FREQUENCY OF DOSAGE	LENGTH OF LIFE FOLLOWING INJECTION OF ORGANISMS	PATHOLOGY	
				GROSS	MICROSCOPIC
T 11	2.0	0.4 Every other day	Killed Twelfth day	Kidneys: Few milinary abscesses. Bile: Culture pos. Urine: Culture neg.	Kidneys: Less acute than T 12. Liver: Occasional degenerate monilia.
T 16	1.5	None	Died Fourteenth day	Generalized milinary moniliasis.	Lungs: Congested and edematous. Kidneys: Hyaline and conidia present in abscesses. Liver: A few conidia.
T 15	1.6	0.45 Every other day	Died Fifteenth day	Generalized milinary moniliasis.	Kidneys: Fewer monilia in abscesses than in T 16. Liver: Like T 16.
T 14	1.75	None	Killed Thirtieth day	Kidneys: Chronic moniliasis. Bile: Culture pos.	Kidneys: Chronic interstitial nephritis. Liver: Isolated monilia.
T 13	1.9	0.45 Every other day	Killed Thirtieth day	No lesions. Bile: Culture pos.	Kidneys: Negative. Liver: Isolated monilia.
T 19	6.8	None	Died First day	Lungs: Congested; numerous petechial hemorrhages. Kidneys: Congested. Heart: Dilated.	Lungs: Thrombosis. Kidneys: Many monilia cells. Liver: Monilia present.
T 20	6.1	0.4 Daily subcutan.	Killed Fourth day	Generalized milinary moniliasis.	Kidneys: Acute reaction, monilia with hyaline.
T 21	7.2	0.5 Daily	Killed Fourth day	Like T 20.	

EFFECT OF THYMOL INFECTIONS CAUSED BY *MONILIA ALBICANS*

The experiments concerned with the effect of thymol on rabbit moniliases were conducted as follows:

For infection purposes, culture 4,135 of *Monilia albicans* was grown in 1 per cent glucose broth for thirty-six hours. It was then centrifuged and taken up in saline solution. The organisms were counted by means of a blood counting chamber. Volumes calculated to hold the necessary numbers of organisms were injected intravenously.

A dose of thymol in olive oil was administered by the stomach tube method, previously described, one hour before the organisms were to be injected. In some cases the dose was repeated at one- or two-day intervals.

Autopsies were performed as indicated in Table III. Cultures on malt agar were made from bile and urine. Kidney and liver tissue, and sometimes pieces of other organs, were fixed, sectioned, and stained for the study of microscopic pathology. Hematoxylin and eosin were used on some sections, and a Gram stain, to reveal the presence of *Monilia*, was used on other sections of the same tissue.

Except for the three animals listed at the end of Table III, results are given in an order based upon the length of life of the rabbits following injection. The last three animals were given massive doses to determine whether thymol had any effect upon a very rapidly fatal infection. The results were not striking enough to be conclusive.

Apart from the inconsistency in the virulence of the culture it seems clear that the administration of the thymol one hour prior to the injection of the organisms had no regularly beneficial effect. While there appeared to have been some diminution in the number and severity of lesions in the cases of rabbits T 7, 9, 11, 13, and 31, as compared with their controls, nevertheless rabbits T 23, 27, 32, and 33 showed more severe moniliasis than their controls. Variability in size of inoculum did not seem to be responsible for the differences in reaction of the animals; both those seemingly benefited by thymol and those definitely not benefited were infected by doses of diverse sizes. The frequency of dosage also seemed to make no difference. The cause of the variation in behavior is, therefore, not known. In our work thymol has not proved to be an effective therapeutic agent against experimental moniliasis.

## SUMMARY

1. Investigations on the cidal properties of thymol for *Monilia* species showed that a saturated aqueous solution at 25° C. would kill all cultures in less than five minutes and most of them in less than one minute.

2. The presence of NaCl in a concentration of 0.9 per cent in the thymol solution had little or no effect upon the cidal action of the drug.

3. Thymol in a concentration of 1:5,000 in glucose broth inhibited for two weeks the growth of 18 of the 19 cultures investigated, but did not kill them. Growth of the remaining culture was inhibited for one week. The same concentration incorporated into malt agar inhibited growth during a shorter

period, ranging from forty-eight hours to four days. A concentration of 1:4,000 was completely inhibitory for two weeks in both media, both at pH 6.0 and pH 7.8.

4. Thymol in 1:5,000 concentration in rabbit serum showed no tendency to inhibit the multiplication of the three species of *Monilia*.

5. Rabbits are apparently able to tolerate without serious ill effects daily doses of thymol amounting to 0.4 or 0.5 gram per kilogram of body weight, administered in oil through a stomach tube. Absorption is apparently complete, since only negligible amounts can be recovered from the feces. Appreciable amounts can be recovered by the steam distillation of the acidified urine. Demonstrable quantities can also be recovered from the blood and liver four hours after administration of the dose.

6. Giving thymol in the way described above, one hour previous to the intravenous injection of *Monilia albicans* and at daily or bidaily intervals thereafter, had no consistently inhibitory effect upon the progress of the infection.

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# A STUDY OF SOME OF THE FACTORS INFLUENCING THE SEDIMENTATION TEST\*

FRED BOERNER, V.M.D., AND HARRY F. FLIPPIN, B.S., M.D., PHILADELPHIA, PA.  
WITH THE TECHNICAL ASSISTANCE OF REBECCA GOODMAN, L.T.

THE purpose of this work is to investigate further some of the inherent variable factors that are known to influence the sedimentation rate of the erythrocytes. In this investigation the concentration of the erythrocytes, the position of the sedimentation tube, and the time interval between the collection of blood and the starting of the test are considered. The extent to which these factors influence the sinking rate of the cells is determined and the methods that were used to make the extrinsic condition normal are presented.

The selection of the Westergren tube for this investigation was made because it was believed that by its length of 200 millimeters slight variations became more noticeable. The greater length of this tube allows the cells to sink a greater depth thereby prolonging the first phase of the sedimentation phenomenon. This assumption is based upon the generally accepted interpretations of the various parts of the sedimentation curve. Indeed all tests showing normal or abnormal rates will, if carried over a sufficient time, show some sort of curve when the readings are made at intervals and plotted against time. This curve is usually divided into two phases, the first being that part of the curve representing the rapid and orderly sinking of the cells, and the second, a progressive slowing of the rate due to the concentration and packing of the cells in the bottom of the tube. It is the general belief that the first phase is the significant one in determining the actual rate of cell descent. For this reason the sedimentation index should be determined during or at the end of this phase. By using the Westergren tube with a column of 200 mm., we rarely find the first phase to end before the end of the first hour.

The choice of a saturated solution of potassium oxalate as an anticoagulant was made because the use of one drop of it (necessary to prevent the coagulation of 8 to 10 c.c. of blood) rendered the dilution factor negligible. According to Rubin and Smith<sup>1</sup> the cell volume suffers less alteration in oxalate than in citrate. Osgood and Haskins<sup>2</sup> have recommended the amount of oxalate used above. In a series of preliminary comparative tests there was no appreciable difference between potassium oxalate and 3.8 per cent sodium citrate.

## THE CORRECTION OF THE CELL CONCENTRATION

Using the method and suggestion of Walton<sup>3</sup> a series of sedimentation tests was conducted before and after correcting the erythrocyte concentration. The method calls for 8 to 10 c.c. of blood obtained by vein puncture. The blood is

\*From the Laboratory of the Graduate Hospital and the William Pepper Laboratory of Clinical Medicine.

Received for publication, July 26, 1934.

immediately placed in a test tube containing one drop of a saturated solution of potassium oxalate. After thoroughly mixing the contents, an erythrocyte count is done. If the concentration of cells is between 4.5 and 5.5 million per c. mm. no correction is made as the cell concentration is considered normal. If, however, the count is below 4.5 or above 5.5 million, a correction is made to bring

TABLE I

COMPARISON OF SEDIMENTATION BEFORE AND AFTER CORRECTING THE ERYTHROCYTE CONCENTRATION OF ANEMIC BLOOD

CASE	DIAGNOSIS	RED COUNT ORIGINAL	RED COUNT COR- RECTED	SEDIMENTA- TION RATE* ORIGINAL	SEDIMENTA- TION RATE* CORRECTED
1	Rheumatic fever	4,000,000	5,100,000	70	32
2	Rheumatic fever	3,500,000	5,000,000	68	34
3	Pregnancy	3,000,000	4,800,000	76	30
4	Coronary disease	4,250,000	5,200,000	62	32
5	Diabetes	4,000,000	5,250,000	42	18
6	Nephritis	3,000,000	4,800,000	38	12
7	Nephritis	3,200,000	5,100,000	98	50
8	Vincent's angina	4,150,000	5,000,000	105	86
9	Coronary disease	4,000,000	5,100,000	28	4
10	Cardiac disease	4,000,000	4,850,000	10	2
11	Peptic ulcer	3,200,000	5,100,000	48	8
12	Postpartum	3,450,000	5,050,000	80	35
13	Postpartum	3,500,000	5,000,000	110	30
14	Pregnancy	3,600,000	5,100,000	80	28
15	Pregnancy	3,800,000	4,900,000	55	18
16	Osteomyelitis	3,500,000	5,100,000	88	40
17	Brain abscess	4,000,000	5,000,000	74	30
18	Coronary disease	3,700,000	4,900,000	86	58
19	Rheumatic fever	4,000,000	5,200,000	90	42
20	Pelvic inflammatory disease	4,100,000	5,000,000	120	82
21	Urethral stricture	4,000,000	4,900,000	125	90
22	Tuberculosis	3,700,000	5,200,000	84	54
23	Cardiac disease	4,225,000	5,000,000	8	4
24	Pelvic inflammatory disease	3,800,000	5,150,000	130	62
25	Osteomyelitis	3,250,000	5,000,000	102	58
26	Bronchopneumonia	3,890,000	5,300,000	70	3
27	Appendiceal abscess	4,000,000	5,020,000	125	60
28	Dermoid cyst	4,100,000	4,840,000	80	30
29	Carcinoma of lip	3,850,000	4,850,000	120	90
30	Burns, 1st and 2nd degree	3,830,000	4,950,000	94	18
31	Subacute P. I. D.	3,410,000	5,250,000	99	35
32	Chaneroid	3,480,000	4,850,000	103	6
33	Ovarian cyst	3,750,000	5,050,000	50	2
34	Carcinoma of ovary	3,690,000	5,300,000	117	22
35	Acute P. I. D.	3,840,000	5,000,000	113	77
36	Hypertension	3,200,000	5,250,000	36	3
37	Undiagnosed	3,130,000	5,100,000	31	1
38	Undiagnosed	3,750,000	5,100,000	31	1
39	Undiagnosed	3,350,000	5,050,000	87	1
40	Fibroid uterus	3,230,000	4,800,000	78	44
41	P. I. D.	3,800,000	5,350,000	105	62
42	Retroverted uterus	4,070,000	5,100,000	26	2
43	Undiagnosed	3,370,000	4,750,000	38	1
44	Undiagnosed	3,620,000	4,900,000	24	2
45	Pellagra	3,650,000	4,850,000	53	10
46	Chronic nephritis	2,700,000	5,200,000	118	5
47	Arthritis	3,610,000	5,300,000	113	59
48	Metritis	3,460,000	4,940,000	52	4
49	Fibroid uterus	1,900,000	5,000,000	98	2
50	Nephrosis	3,450,000	4,780,000	125	91

\*Sedimentation rate = the amount of sedimentation at the end of one hour expressed in millimeters.

the concentration of the cells within the above stated limits. This is done simply by removing enough plasma to raise the concentration of the cells to the desired range in bloods having cell counts below the normal range, or adding enough plasma to lower a cell concentration of higher than normal range.

The technique of adjusting the cell concentration to the normal range is carried out by using 5 c.c. of the sample. This blood is centrifuged sufficiently to clear enough plasma to make the correction. The amount of plasma to be removed or added depends upon the cell count in millions per cubic millimeter.

TABLE II  
THE SEDIMENTATION RATE WITH NORMAL BLOOD

NO.	SEX	R.B.C.	SEDIMENTATION RATE
1	Female	5,500,000	3
2	Female	5,050,000	3
3	Female	5,200,000	4
4	Female	5,170,000	4
5	Female	4,750,000	2
6	Female	4,700,000	4
7	Female	5,800,000	3
8	Female	4,850,000	2
9	Female	4,490,000	4
10	Female	5,100,000	3
11	Female	5,000,000	4
12	Female	4,850,000	3
13	Female	4,750,000	3
14	Female	5,100,000	4
15	Female	5,100,000	4
16	Female	5,200,000	3
17	Female	4,900,000	3
18	Female	5,000,000	5
19	Female	5,200,000	4
20	Female	4,750,000	4
21	Female	5,125,000	4
22	Female	4,750,000	5
23	Female	5,150,000	3
24	Female	5,240,000	4
25	Female	5,000,000	3
26	Male	5,250,000	3
27	Male	5,250,000	2
28	Male	5,300,000	1
29	Male	5,400,000	1
30	Male	5,200,000	1
31	Male	5,280,000	3
32	Male	5,350,000	1
33	Male	5,450,000	1
34	Male	4,700,000	1
35	Male	5,350,000	1
36	Male	5,550,000	1
37	Male	5,050,000	1
38	Male	5,350,000	1
39	Male	4,900,000	1
40	Male	5,350,000	1
41	Male	5,300,000	1
42	Male	5,200,000	3
43	Male	5,100,000	1
44	Male	5,250,000	3
45	Male	5,000,000	4
46	Male	4,850,000	2
47	Male	5,125,000	3
48	Male	4,800,000	2
49	Male	4,750,000	2
50	Male	5,100,000	1

If the count is below 4.5 million, enough plasma is removed so that the remaining volume in cubic centimeters equals the number of the millions in the count, e.g., if the blood is 3.5 million, 1.5 c.c. of plasma are removed, leaving a volume of 3.5 c.c. Or if the count happens to be 6.0 million, 1 c.c. of plasma from another sample is added to make a total volume of 6 c.c. After this adjustment the sample is well mixed and a count is made to check the correction. Two sedimentation tubes are used. One contains the uncorrected, the other the corrected sample. They are suspended in a vertical position. The readings are made simultaneously at the end of one hour. In Table I are the results of the observations on 50 patients having secondary anemia from various causes. These results should be compared with the observations on 50 normal individuals (25 females and 25 males) whose cell concentrations were considered to be in the normal range (see Table II).

#### METHOD OF PLACING THE WESTERGREN TUBE IN A VERTICAL POSITION

All the methods describing the test state that the sedimentation tube should be placed in an upright position. However it is felt that too little emphasis has been given this factor. Ponder<sup>4</sup> has demonstrated that a divergence from the

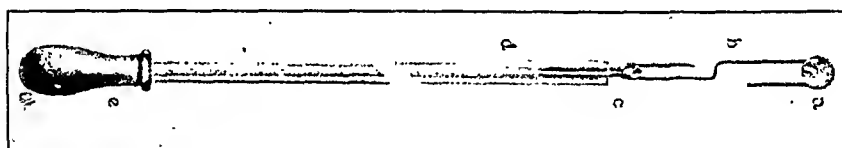


Fig. 1.—A drawing of the Westergren tube suspended from a thumb tack. *a*, thumb tack; *b*, bent gem paper clip serving as a hook; *c*, cotter pin; *d*, Westergren tube; *e*, the rubber bulb with a window.

vertical position of 7.5 degrees will almost double the sedimentation rate when using a tube of 200 mm. length or more. Some of the racks especially made for holding the tubes are not constructed to insure a perfect vertical position. After the tubes are placed in the rack, designed to hold them vertically, the rack should then be placed upon a surface which is perfectly horizontal. To insist upon these details involves considerable time and pains upon the part of the operator. If adjustments are made to this end, they may not be reliable unless precision with proper instruments is used. This, however, is not practical when a perfect vertical position of the tube may be had by simply suspending it so that it hangs freely. A method was devised in the laboratory of the Post Graduate Hospital and is exceedingly simple. The vertical position is accomplished by suspending the sedimentation tube from a thumb tack by means of a small hook, made from a Gem paper clip, and a snugly fitting cotter pin inserted into the upper end of the tube. After the tube has been filled to the 200 mm. mark with blood, the lower end is sealed by inserting it into a small rubber bulb. To facilitate slipping the bulb into position without disturbing the level of the column of the blood, it is moistened and a small window is cut into the wall near the blind end (see Fig. 1).



## TIME INTERVAL BETWEEN COLLECTING BLOOD AND STARTING THE TEST

Cooper,<sup>5</sup> Osgood and Haskins,<sup>2</sup> and others state that the test should be done as soon as possible after the blood has been collected. The limits of delay have been variously put from three to ten hours. It was deemed advisable to determine what extent various time intervals would influence the results as there was always some delay due to time consumed in correcting the erythrocyte concentration. The influence of standing was determined by obtaining sufficient blood from each of 25 patients to conduct six different tests, and from 10 patients to perform four tests. One test was started immediately, the others, one, two, three, four, and five hours after collection. The blood was allowed to remain in the test tube in which it was collected during the above delay periods. Before each test was started the blood was thoroughly mixed by shaking. Then the Westergren tube was filled to the mark, sealed with the rubber bulb and suspended so that it hung freely. The rate was determined by reading at the end of one hour. Table III contains the data from these experiments.

TABLE III  
EFFECT OF TIME INTERVAL BETWEEN OBTAINING BLOOD AND STARTING TEST UPON SEDIMENTATION RATE

CASE	DIAGNOSIS	IMMEDIATELY	AFTER FIRST HR.	AFTER SECOND HR.	AFTER THIRD HR.	AFTER FOURTH HR.	AFTER FIFTH HR.
1	Postpartum	76	82	75	82	64	60
2	Postpartum	56	50	36	34	22	20
3	Postpartum	62	50	30	23	10	10
4	Eclampsia	140	142	142	136	120	118
5	Peptic ulcer	80	65	54	58	60	35
6	Coronary disease	30	15	5	4	2	2
7	Rheumatic fever	40	25	18	6	5	2
8	Ulcerative colitis	110	105	102	80	100	45
9	Femoral thrombosis	86	80	24	5	3	3
10	Chronic lymphatic leucemia	104	96	94	70	82	56
11	Coronary disease	4	3	2	1	1	1
12	Hodgkin's disease	25	18	10	4	3	2
13	Perinephric abscess	64	50	40	36	20	18
14	Inguinal hernia	60	52	50	52	40	30
15	Inguinal hernia	70	82	80	50	28	24
16	Renal stone	54	52	30	18	14	12
17	Rheumatic fever	42	40	34	18	12	12
18	Pregnancy	60	54	50	36	30	22
19	Pregnancy	56	46	42	35	28	24
20	Coronary disease	18	18	15	12	4	6
21	Osteomyelitis	102	100	98	96	80	55
22	Tuberculosis	88	86	86	80	70	50
23	Pelvic inflammatory disease	120	124	118	110	100	88
24	Pelvic inflammatory disease	102	100	100	90	104	78
25	Brain tumor	42	38	38	30	28	24
26	Undiagnosed	12	10	4	2		
27	Undiagnosed	97	95	81	5		
28	Undiagnosed	26	26	16	11		
29	Undiagnosed	81	81	80	67		
30	Undiagnosed	120	117	108	106		
31	Undiagnosed	55	42	35	20		
32	Undiagnosed	84	83	47	15		
33	Undiagnosed	65	63	20	10		
34	Undiagnosed	110	99	97	38		
35	Undiagnosed	109	107	81	55		

## DISCUSSION

The cases selected to observe the cell concentration variable were a group of 50 patients exhibiting secondary anemia from various causes. The purpose for this is to ascertain and study the change in the rate after adjusting the cells to normal range. Of the 50 cases giving abnormal sedimentation rates, 14 were normal after the cells were adjusted to normal limits. In all cases (see Table I) the sedimentation rate was lower after the cell concentration was adjusted to normal range. The differences between corrected and uncorrected bloods are not in any way proportional, as a group, to the degree of anemia. It follows from this finding that a mathematical formula, based upon a preliminary cell count and subsequent rate, designed to anticipate and correct the anemia factor is unreliable. These results are in harmony with those of Walton<sup>3</sup> and others. If the corrected blood in our experiments is to be considered identical in every respect to an unmolested sample having a normal cell concentration, here is evidence showing that the sinking rate of solids in a liquid is partly influenced by the number present.

The sedimentation rate obtained with the blood of 50 normal individuals ranges from 2 to 4 mm. for females and from 1 to 3 mm. for males. Various normals are reported ranging from 5 to as high as 26 mm. From our results and those of Walton, there is evidence suggesting that the higher normal rates reported by others were due either to a low erythrocyte concentration, or the sedimentation tubes were not in a vertical position during the test.

Relative to the time interval between the collection of blood and starting the test, the evidence from our data indicates strongly that after one hour the rates become progressively slower and directly proportional to the length of delay. This is an interesting phenomenon which cannot be reliably ascertained unless other variables such as we have described are reduced to negligible. It also indicates, when the test is carefully performed in every other respect, that the inherent slowing due to delay may mask an otherwise increased rate. It is best to perform the test within one hour after collection as tests started after two or three hours' delay showed slight to marked effect upon the sedimentation rate (see Table III).

## CONCLUSIONS

1. Observations upon a few of the inherent variable factors influencing the sedimentation rate are discussed.
2. Correcting the erythrocyte concentration before conducting the sedimentation test, as suggested by Walton, greatly enhances the value of the test by putting the blood in a condition comparable with normals, allowing other reasons for variation of sedimentation to show themselves.
3. The position of the sedimentation tube during the test is discussed, and a simple method is described for the placing of the Westergren tube in a vertical position.
4. Sedimentation tests performed after a delay of two, three, or more hours show a slower sinking time than when made immediately.

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OBSERVATIONS RELATING TO THE SPECIFICITY OF THE  
DICK TEST\*

H. E. SMILEY, M.D., PROVIDENCE, R. I.

FOR the last four years all nurses admitted to training at the Charles V. Chapin Hospital have been "Dick tested" on entry. All those showing a positive reaction have been recommended for immunization. During the period from Dec. 3, 1929 to Nov. 22, 1933, 2,018 nurses were "Dick tested." Of these, 722 (35.8 per cent) gave a positive reaction, 173 (8.6 per cent) gave a doubtful reaction, and 1,123 (55.6 per cent) gave a negative reaction.

Of the 722 giving a positive Dick test, 87 began the immunization course. In a great many instances severe reactions followed so that the course in these cases was stopped. Table I shows in detail the record of the re-Dick tests made two weeks after the last inoculation. Summarizing this table, it can be seen that of the 87 nurses immunized (whether complete course or not), 36 (41.5 per cent) gave a positive re-Dick test; 13 (14.9 per cent) gave a doubtful re-Dick test; and 29 (33.3 per cent) gave a negative re-Dick test. Nine (10.3 per cent) were not tested.

TABLE I  
SUMMARY OF RE-DICK TESTS FROM DEC. 3, 1929, TO NOV. 22, 1933

NUMBER OF NURSES IMMUNIZED	NUMBER OF INOCULATIONS RECEIVED	RE-DICK TEST, TWO WEEKS AFTER LAST INOCULATION			
		POSITIVE	DOUBTFUL	NEGATIVE	NOT TESTED
39	5	9	5	23	3
12	4	7	3	2	
9	3	5	2	2	
8	2	2	2	2	2
19	1	13	1	1	4
Total 87		36 (41.5%)	13 (14.9%)	29 (33.3%)	9 (10.3%)

Dick tests are difficult to read. The Dicks state that the faintest reddening, 1 cm. or over in any diameter, indicates some degree of susceptibility to scarlet fever. We have followed this rule in reading our Dick tests. If the area of reddening is 0.5 cm. in diameter, it is called +, 1 cm. in diameter ++,

\*Received for publication, July 2, 1934.

1.5 cm. in diameter ++, and 1.8 cm. or greater ++++. All readings ++, +++, and ++++ are considered positive; + or smaller are called doubtful and no area of reddening about the needle puncture wound negative.

It seemed to us that the percentage of positive re-Dick tests was higher than was to be expected, and that suitable controls should be used.

The original Dick technic calls for the use of the Dick test solution without any control. Inasmuch as most immunologic tests are checked by controls, we began controlling the Dick test with the same solution heated to different temperatures. Since August, 1933, we have controlled every nurse's Dick test with the same solution heated to destroy the toxin. The results of the controlled tests showed that it made little difference whether the toxin was destroyed by boiling temperature for one hour or autoclaving for two hours; the control test oftentimes was as positive as the test itself. In all, 116 nurses were tested. Of these, 70 nurses gave a positive Dick test, 33 gave a doubtful reaction and 13 gave a negative reaction. Of the 70 nurses giving a positive Dick test, the control tests were as follows: positive 46 (65.7 per cent), doubtful 21 (30 per cent), negative 3 (4.3 per cent). Of the 33 nurses giving a doubtful Dick test, the controls were as follows: positive 7 (21.2 per cent), doubtful 23 (69.7 per cent), negative 3 (9.1 per cent). Of the 13 nurses giving a negative Dick test, the controls were as follows: positive 1 (7.7 per cent), doubtful 5 (38.5 per cent), negative 7 (53.8 per cent). To put it another way, out of 116 control tests, all of which should have been negative, 54 (46.5 per cent) proved positive, 49 (42.3 per cent) proved doubtful and only 13 (11.2 per cent) proved negative. These results are very disconcerting and only tend to discredit the value of the Dick test. When a control gives the same reading as, or greater than, the test reading it throws a great deal of doubt upon the specificity of the reaction. Any scientific test, such as the Dick test, should be so controlled as to have the two solutions similar in all respects but one, and that one should be the factor in question, in this case the scarlet fever toxin.

When it became so apparent that there was a question as to the specificity of the Dick test a correspondence was begun with the director of a very reliable biologic laboratory that is an authorized manufacturer of the Dick toxin under the Dick patent. The early part of our correspondence dealt with methods of destroying the Dick toxin, but it soon became evident that so far as this point was concerned we were "barking up the wrong tree." The trouble was evidently not with the toxin. Our attention was then directed to the Dick test solution itself. The director furnished us with the formula of the Dick test solution as put out by his company. This solution consists of a buffered salt solution, containing phenol for a preservative, and scarlet fever toxin. Experiments were commenced using various controls and 12 laboratory assistants were used as subjects. The object of the experiments was an attempt to find out why the control solution produced a reaction.

Preliminary experiments seemed to indicate definitely that the trouble lay in the diluent, because tests made using the diluent without the toxin solution gave positive reactions. In analyzing these results we attacked the problem from 3 angles: (1) was the reaction due to the presence of phenol

which might act as an irritant? (2) was the reaction due to the pH of the buffer diluent? and (3) was the reaction due to irritation by potassium ions? Table II lists the experiments tried out, investigating each of these questionable points.

TABLE II

0.3% PHENOL IN H <sub>2</sub> O	BUFFER WITH PHENOL pH 7	BUFFER WITHOUT PHENOL pH 7			BUFFER WITH-OUT PHENOL pH 7.2	BUFFER WITH-OUT PHENOL pH 7.3	BUFFER WITH-OUT PHENOL pH 7.4		BUFFER WITH-OUT PHENOL pH 7.6	KCl 1.14% ISO-TONIC	NaCl ISO-TONIC
+	0	±	++	+++	+++	+++	+	+	++	++	0
0	+	+	+	+++	+++	++++	0	++	+	+++	0
±	±	++	++	+	+	+++	±	±	±	++	±
±	+	++	++++	+	+	+	++	+	++	++	0
0	+	+++	+	++	++	++	++	+	++	++	0
0	++	+++	++++	+	++	++	++	++	++	+++	0
+	+	+	+++	+++	++	++	+	++	++	+++	0
0	+	+	++	++	++	++	+	++	++	++	±
+	+	++	++	+	+	+	+	+	+	++	0
±	±	++	++	+	+	+	±	++	++	++	±
0	+	++	+++	++	++	++	++	++	++	++	+
0	+	+++	++	++	++	++	+	+++	+++	+++	0

It is interesting to note that the phenol solution alone gave practically no reaction, and also that the buffer solution plus phenol gave less severe reactions than the buffer solution without phenol. Our interpretation of this is that the phenol acts not as an irritant but as an anesthetic, by toning down the reaction. As to the question whether the reaction might be due to the pH of the solutions injected, seven different buffer solutions were prepared varying only in pH. The reaction of the Dick test solution, as approved by the Scarlet Fever Committee, has a pH of 7. This is apparently more acid than normal tissue fluids and the experiments cited above, were made to determine whether or not varying the pH affected the reaction. An analysis of the results given above tends to indicate that the pH, within these limits at least, has little or nothing to do with the reaction. In the other test experiment to determine whether or not potassium ions are irritating, an isotonic potassium chloride solution was substituted for the Dick diluent. This solution, as shown in the table, gives strongly positive reactions in nearly every instance. In this case, however, the concentration of potassium ions was much greater than that in the buffer solutions, so the experiment was repeated using the isotonic solution diluted 1:30 with distilled water, which made the concentration of potassium ions approximately the same as in the saline buffer used in the diluted Dick toxin. While all those tested remarked about the smarting, stinging sensation immediately experienced, no redness was present the next day, a result which tended to eliminate the potassium ions from suspicion.

In all our experiments one fact has stood out prominently, namely the inconstancy of the results; in other words, duplicate, similar tests made on the same individual, after varying time intervals, gave different readings. Whether the initial Dick test in some way modifies the skin field, either by sensitizing the skin area or by producing a local immunity, is a problem hard

to solve. Of course, if only one test is made on an individual, and if that test is not controlled, no comparison can be made and the resulting reading of that solitary test stands unchallenged. It is only when controlled tests are made and when re-Dick tests are done that the inconstant and varying readings are disclosed which obscure the interpretations.

At this point in our studies it seemed perfectly clear that pseudoreactions occur using the standard Dick toxin solution. Further experiments were done using the standard solution as purchased on the open market from an authorized laboratory. A duplicate amount of Dick toxin solution was purchased and this was autoclaved at 15 pounds pressure for one hour to destroy the toxin. Dick tests were performed on 95 nurses using these two solutions. Of the 95 tests, 22 (23.1 per cent) gave negative results with both the standard solution and with its control; 15 (15.7 per cent) showed positive readings with the standard solution and negative readings with the heated control; 16 (16.6 per cent) gave readings in the control which were weaker than the test readings, which would tend to modify the degree of positivity of the test solution; and lastly, 42 (44.2 per cent) control tests gave the same reading as or greater than the standard. In other words 44 per cent gave false positives.\*

Another experiment was done comparing the standard Dick toxin solution with the buffer solution containing no toxin. Fifty nurses were tested. Of these, 14 (28 per cent) gave negative readings with both solutions; 14 (28 per cent) showed the control to be negative while the test solution was positive; 8 (16 per cent) gave weaker readings with the control than with the test inoculation, and 14 (28 per cent) showed readings in the control equal to or greater than the standard reading. In other words 28 per cent of the readings were false positives; apparently in these cases due to the influence of the buffer solution alone.

Inasmuch as the Dick test is used as a criterion to determine susceptibility to scarlet fever and also as an index of successful immunization, it is very essential that the test should be reliable so that its interpretation may be of some value.

A study of all the experiments performed, elinched by these final two experiments, seems to demonstrate clearly the following conclusions:

1. Dick tests should be controlled using the same solution heated at 15 pounds pressure in the autoclave for one hour.
2. Pseudoreactions occur which in some instances are apparently due to the diluent while in other instances the reason is not so apparent.

I wish to acknowledge the generous cooperation furnished me by the director of the Biological Laboratory in this study. Also I wish to acknowledge the assistance offered me by my associates with whom the problems arising in the course of this study were freely discussed.

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\*Since this paper was submitted for publication (July 2) 295 more cases have been tested and the results tend to modify the percentages given above. The final classification of these 295 cases is as follows: true positive reactions 73 (24.7%), true negative reactions 128 (43.2%), modified positive reactions 40 (13.5%), and false positive reactions 55 (18.6%).

## THE EFFECTS OF BLOOD TRANSFUSIONS ON DONORS\*

JAMES W. MARTIN, M.S., M.D., AND JOHN T. MYERS, M.D., PH.D., OMAHA, NEB.

SINCE blood transfusion is a frequent therapeutic measure, the effects of blood loss deserve consideration. Giffin and Haines,<sup>1</sup> from a study of donors made at indefinite intervals after blood donations, concluded that blood letting every four or five weeks was not harmful; that women developed anemia more rapidly and recovered more slowly than men; that 27 per cent felt improved in health; that 50 per cent gained weight and that studies of blood volume showed no change in plasma and cell volume.

A study of effects from blood loss in donors has been made by Jones, Widing and Nelson.<sup>2</sup> In their study of 175 transfusions, hemoglobin, erythrocytes, leucocytes, blood platelets, clotting time, volume index, vital capacity, and weight were estimated before and after each transfusion.

In a series of 52 transfusions given by ten donors, we have made the following observations:

1. The amount of blood given at each transfusion was noted.
2. The erythrocyte and leucocyte counts and hemoglobin estimation were made immediately before and after each transfusion, three, and six hours thereafter, then once daily until it returned to normal. The Sahli method (14.7 mg. per 100 c.c. of blood) of hemoglobin determination was used.
3. Reticulocyte counts were made before each transfusion and daily until the original number of erythrocytes was restored.
4. Each donor was weighed before and directly after each transfusion and daily for from four days to two weeks.
5. Bleeding and clotting times were taken once daily on the first patients in the series, but, as no change could be detected, this was discontinued.
6. Blood pressure was taken before and after each transfusion and on the following day.

CASE 1.—A medical student, aged twenty-six, first served as a donor Feb. 22, 1928 and, on May 8, 1933, served the twenty-third time. Five hundred cubic centimeters were given on twenty occasions, 300 on two, and 550 on one. Before his first transfusion, his erythrocyte count was 5,100,000 and hemoglobin 90. On May 15, 1933, it was 5,400,000 and hemoglobin 96. The erythrocyte count taken each time directly after the transfusion showed no decrease. After three hours, there was usually a drop of about 250,000 and, at six hours, 310,000 which was the average reduction of erythrocytes for this donor when 500 c.c. was taken. This would suggest that it requires four to six hours for the blood volume loss to be replaced by fluids from the tissues.

The reticulocyte count increased on an average of 11 per cent the day following and gradually decreased to its prior figure as the original erythrocyte count was regained.

\*From the Department of Pathology and Bacteriology, University of Nebraska, College of Medicine.

Received for publication, July 3, 1934.

The decrease in hemoglobin averaged 5.2 per cent, reaching its lowest at six hours. There was no appreciable change in the color index.

The leucocyte reduction was in proportion to the reduction in erythrocytes.

The blood pressure dropped between six and ten millimeters but was regained in six hours, probably as soon as the blood volume was restored.

When this donor first started giving transfusions, six days were required to regain completely the original erythrocyte count. After repeated transfusions, about one every three or four months, it was always regained by the fourth day, perhaps due to a stimulation of the hemopoietic system. This was noticeable after the twelfth transfusion. His erythrocyte count one week after the twenty-third transfusion was more than it was before the first.

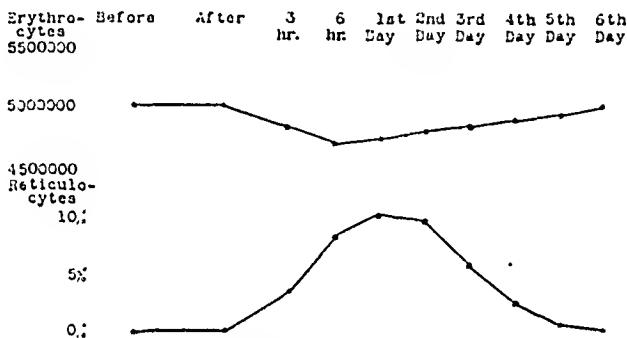


Chart 1.—The reticulocyte and erythrocyte counts of Case 1 before and after the first transfusion on Feb. 22, 1928.

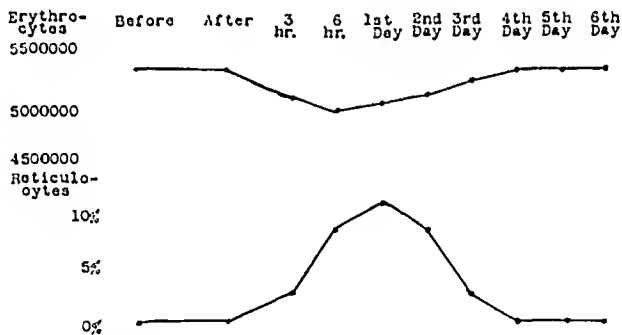


Chart 2.—The reticulocyte and erythrocyte counts of Case 1 before and after the twenty-third transfusion on May 8, 1933.

The weight loss after each transfusion averaged one and one-half pounds which was regained each time by the second day. In this case and in others reported in this paper, there was no gain in weight, although Giffin and Haines<sup>1</sup> observed a gain after repeated transfusions. Jones, Widing and Nelson<sup>2</sup> report the same, one donor showing a gain of thirteen pounds. When there is a gain in weight, one wonders whether it is not due to the fact that the donor believes he should eat heartily to gain back what he lost and, consequently, adds weight because of a greatly increased food intake. Following the fifteenth transfusion, on Aug. 7, 1931, the above donor ate three hearty meals each day, took plenty of fluids and drank two malted milks per day. He gained five and one-half pounds in eight days and then, on an ordinary diet, dropped to his normal weight in one week.

This donor could feel no ill effects from the transfusion. Frequently, he returned to his work within an hour after the blood was taken and could not notice being weak.



TABLE I. THE BLOOD FINDINGS IN CASE 1, BEFORE AND DIRECTLY AFTER EACH TRANSFUSION

TRANS- FUSION DATE		AMOUNT	ERYTHRO- CYTES	HEMO- GLO- BIN	C. I.	RETICU- LOCYTES	LEUCO- CYTES	WT.	BLEED- ING CLOT- TING	BLOOD PRES- SURE
1. 2/22/28	Before	500 c.c.	5,100,000	85	0.83	0.2%	8,700	156.0	4-2	120/82
	After		4,750,000	80	0.84		7,200	154.5	4-2	116/75
2. 3/21/28	Before	850 c.c.	5,100,000	87	0.85	0.1%	7,800	156.2	4-3	122/81
	After		4,200,000	70	0.83		7,200	154.0	4-3	110/62
3. 5/15/28	Before	500 c.c.	5,150,000	87	0.84	0.2%	7,600	156.4	4-3	120/79
	After		4,500,000	83	0.92		7,300	155.0	4-3	117/75
4. 8/25/28	Before	500 c.c.	4,900,000	86	0.85	0.2%	7,800	154.0		120/80
	After		4,600,000	82	0.88		7,400	153.0		116/75
5. 12/21/28	Before	500 c.c.	5,200,000	87	0.83	0.1%	7,700	150.0		120/82
	After		4,970,000	84	0.85		7,400	148.7		117/76
6. 5/ 2/29	Before	500 c.c.	5,200,000	89	0.85	0.2%	7,600	152.0		120/80
	After		4,850,000	85	0.87		7,400	151.0		116/72
7. 6/ 1/29	Before	500 c.c.	5,220,000	88	0.85	0.1%	7,600	150.0		121/80
	After		4,900,000	85	0.86		7,300	149.5		115/70
8. 9/14/29	Before	500 c.c.	5,200,000	89	0.85	0.3%	7,700	151.0		122/81
	After		4,900,000	85	0.86		7,400	149.7		116/73
9. 3/29/30	Before	500 c.c.	5,220,000	90	0.86	0.2%	7,600	152.0		121/82
	After		4,900,000	86	0.85		7,400	151.5		115/72
10. 4/18/30	Before	500 c.c.	5,200,000	90	0.86	0.1%	7,800	152.6		120/80
	After		4,850,000	84	0.86		7,500	150.8		116/75
11. 8/26/30	Before	500 c.c.	5,210,000	89	0.87	0.2%	7,700	151.5		121/80
	After		4,986,000	85	0.86		7,400	150.0		117/70
12. 11/11/30	Before	500 c.c.	5,220,000	90	0.86	0.3%	7,700	150.0		120/82
	After		4,900,000	86	0.85		7,500	148.7		116/73
13. 12/15/30	Before	500 c.c.	5,250,000	91	0.86	0.2%	7,800	152.1		122/82
	After		4,950,000	86	0.85		7,500	150.7		116/72
14. 4/ 6/31	Before	500 c.c.	5,270,000	91	0.86	0.1%	7,600	152.0		120/80
	After		4,990,000	85	0.86		7,300	151.0		117/75
15. 7/10/31	Before	300 c.c.	5,275,000	92	0.83	0.1%	7,600	152.6		122/82
	After		5,000,000	86	0.86		7,400	150.5		120/80
16. 7/28/31	Before	300 c.c.	5,300,000	92	0.83	0.2%	7,700	152.6		122/81
	After		5,075,000	86	0.86		7,300	150.5		120/78
17. 12/16/31	Before	500 c.c.	5,345,000	93	0.87	0.1%	7,800	152.4		120/80
	After		5,000,000	87	0.86		7,400	150.3		116/75
18. 3/14/32	Before	500 c.c.	5,350,000	93	0.87	0.3%	7,700	151.0		121/82
	After		5,025,000	86	0.86		7,500	149.8		116/76
19. 5/12/32	Before	500 c.c.	5,260,000	94	0.89	0.3%	7,800	152.0		122/82
	After		5,030,000	87	0.86		7,500	151.0		117/75
20. 6/ 8/32	Before	500 c.c.	5,375,000	95	0.88	0.2%	7,900	152.5		121/80
	After		5,000,000	90	0.90		7,600	151.0		116/74
21. 9/ 2/32	Before	500 c.c.	5,400,000	96	0.88	0.1%	7,800	152.0		120/81
	After		5,150,000	90	0.89		7,500	150.0		117/75
22. 1/10/32	Before	500 c.c.	5,400,000	96	0.88	0.2%	7,900	152.5		122/80
	After		5,150,000	91	0.88		7,700	151.0		116/75
23. 5/ 8/33	Before	500 c.c.	5,400,000	96	0.88	0.1%	7,900	153.0		122/80
	After		5,025,000	91	0.90		7,700	151.5		117/76

CASE 2.—A medical student, twenty years old, served as a donor the first time May 6, 1928, and Dec. 9, 1933, served the tenth time. The findings in this donor were practically identical with those of the first. There was one slight complication which should be mentioned. The day after giving his sixth transfusion, about a dozen nodules appeared over the pubes and a few on each inner thigh. He complained of itching but there was no other symptom. No treatment was instituted and the nodules disappeared permanently in five days. This could easily have been an intercurrent manifestation.

CASE 3.—A medical student, aged twenty-five, gave six transfusions of 500 c.c. each, in three years. The findings resembled those of Case 1.

CASE 4.—A white male, aged fifty-two, with hypertension gave one transfusion. The findings did not differ from the previous ones except that no fall in pressure could be detected after drawing 500 c.c. of blood.

Table I and Charts 1 and 2 summarize the findings of Case 1. The findings in the other donors were similar to this one.

CASE 5.—A white male, forty-eight years old and a drug addict served as a donor twice. His erythrocyte count was 3,800,000 before giving 500 c.c. of blood. At the end of six hours after the transfusion, it was 3,430,000 and returned very slowly to normal, being down to 3,600,000 after ten days. He then gave a second transfusion of 300 c.c. and his erythrocyte count dropped to 3,400,000. Counts at intervals for six months remained around 3,500,000.

CASE 6.—A female premedical student, eighteen years old served as a donor twice, giving 500 c.c. of blood each time. On both occasions she was three or four days longer than Cases 1 and 2 in regaining her previous erythrocyte count.

CASE 7.—A white male laborer, twenty-seven years old, was a donor twice, giving 500 c.c. the first time and 300 c.c. the second. The findings closely resembled Case 1 with the exception that during the second transfusion when 300 c.c. had been withdrawn, he had what he described as severe cramplike pains in the epigastrium, felt nauseated, and vomited. He felt bad for a day and was discouraged from giving any more.

CASES 8, 9, and 10.—These donors gave one transfusion of 500 c.c. each. No irregular effects were noted.

The question of small or large donations is important. From the observations just considered, it would seem that to give 500 c.c. at three- to four-month intervals is not harmful, but, of course, the possibility of producing undetectable changes must be kept in mind.

It has been shown that frequent bleeding of rats to exhaustion, rapidly depleted the bone marrow.<sup>3, 4, 5</sup> A careful history from prospective donors is important as there are undoubtedly persons with a congenital weakness of the bone marrow who do not stand blood losses well.

#### SUMMARY AND CONCLUSIONS

1. Observations were made on ten donors who had given from one to twenty-three transfusions each.

2. The average reduction in erythrocytes when 500 c.c. of blood was taken was 310,000 per c.mm. which was regained, in most instances, in four to six days.

3. The leucocyte drop was proportional to the erythrocyte decrease.

4. The hemoglobin drop averaged 5.2 per cent when 500 c.c. of blood was taken, using the Sahli method of estimation. There was no appreciable change in the color index.

5. There was an initial weight loss of 1.5 pounds which was regained, in nearly every instance, within two days. No gain in weight was noted.

6. There was no change in bleeding and clotting time.

7. The blood pressure was only slightly reduced and rose again in six hours.

8. Directly after the transfusions, there was no reduction in erythrocytes or hemoglobin but there was a decrease as fluid was taken from the tissues into the circulation, the minimum being reached at the end of six hours.

9. The female donor regained her erythrocytes and hemoglobin more slowly than the male donors.

10. Individuals with a tendency to anemia should, apparently, not be used as donors.

Although a more extensive and complete study of the effects of blood transfusions on donors should be made before reaching definite conclusions, these observations suggest that there are no ill effects, or only slight ones, if 500 c.c. are given at a time and not oftener than once in three months.

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## THE DOCTOR AS INVENTOR\*

EDWARD PODOLSKY, M.D., BROOKLYN, N. Y.

THE doctor has played a very important rôle in invention. He has been the far-seeing pioneer in this as in other fields. Some of his inventions have played a decidedly significant rôle in the lives of all of us. Radio, one of the most popular of all inventions, owes much to the doctor-inventor.

Mahlon Loomis was among the earliest pioneers in the field of radio engineering. Certain facts recently unearthed bring to light the fact that this physician was the first to use an aerial with a kite, the first to discover the phenomenon of wave length, the first to use a battery excited magnetic wave apparatus, though he was ignorant of the real manner of generation of the impulse. He was also the first to discover that the electromagnetic wave was transmitted better on a cloudy day than on a sunny one.

Dr. Loomis was a man of many talents. He was interested in a great many things. Among other things he is credited with the invention of the false tooth plate. This relegated all other dental appliances to the scrap heap. The monstrous collection of wires and levers which formerly served to annoy the toothless is shown in the Alexandra exhibit of the relics of George Washington. Loomis' invention marked a new era in dental science.

Loomis was no ordinary dabbler in electricity; what he did he carried to the limit. Records show that from 1856 to 1859 he buried wires in the ground, charged them by a current from a galvanic battery, and enriched the crops in the family garden at West Springfield, Mass. He invented and patented the alarm thermometer.

The efforts of the Loomis Aerial Telegraph Company, incorporated by a special act of Congress, are now a matter of history. The Chicago Fire and the Black Friday panic of Grant's administration wiped out Loomis' backers and contributed much to breaking the spirits of this pioneer.

There is documentary proof in the United States Patent Office and at various newspaper file rooms that Dr. Loomis discovered the oscillatory current and the application of it to short distance transmission. He used gilded balloons as part of the transmitting and receiving apparatus. Loomis, like so many other pioneers in new and unfamiliar fields, was not understood nor appreciated. He was sixty years old, broken in health, and bent with bitter recollections of his failure to convince others of the magnitude of his project in radio engineering when he died. His patents on his "aerial telegraph" did not expire until seventeen years after their grant by the Patent Office, which was July 30, 1889, three years after his death.

Dr. Loomis was perhaps the least appreciated of the American doctor-inventors, and the one who fared the worst. In other fields of practical invention other American doctors made very important contributions. In the

\*Received for publication, July 5, 1934.

winter of 1844 Dr. John Locke, Professor of Chemistry at the Medical College of Ohio, lectured before its president and officers on magnetic clocks. Dr. Locke showed how the beats of a clock might be repeated and how another clock at a telegraphic distance might be made to move without pendulum or weights. In 1849 the government called Locke to Washington to the Naval Observatory where he constructed the first electro-chronograph. In view of the fact that electric clocks are now in wide use throughout the world, there is no doubt that Dr. Locke has made the greatest contribution to chronography in modern times.

At about the same time another American physician was improving an invention which was to play a very important rôle in every civilized city throughout the world. Around 1848 Dr. William F. Channing applied the principle of electric telegraphy to the fire alarm, and in 1851 placed before the city of Boston a detailed plan for a telegraphic system of transmitting fire alarms. Dr. Channing worked out a complete system of fire alarm boxes, bells, receiving electrical apparatus, etc. Ten thousand dollars was finally appropriated by the city government in order to put his plan into operation. The doctor likened the telegraphic system to the human brain and the nervous system, and drew a strong analogy "between the functions of the motor nerves and the apparatus of the anial system."

The physician has played a rather sinister rôle in inventions for the extermination of human life. This is in direct opposition to his sworn duty to preserve and prolong human life. The modern machine gun had its origins in the inventions of Dr. Richard Jordan Gatling, born in 1818 in North Carolina. Dr. Gatling neglected his practice to devote his time to invention. His earlier inventions were a steam plough and a machine for sowing seeds, but these have not apparently been forgotten. Dr. Gatling is known for his invention of the Gatling gun which was first used in the Civil War. The Gatling gun was the first of the great engines of destruction.

Another doctor who invented a machine to do away with human life was Joseph Ignace Guillotin (1738-1814) whose name would have been long forgotten had he been satisfied merely with practicing medicine. He received his medical education at Rheims and at Paris, where he practiced for a few years. But it was politics he was really interested in, and in 1789 he was elected to the Constituent Assembly. In that very same year he brought forward a proposition that all capital punishment should be by decapitation and by a specially devised machine. He wanted death to be swift and painless. Two years later a law was passed that everyone condemned to death in France should be decapitated. Dr. Guillotin invented the machine to accomplish this decapitation. It was at first called the lousette, but later came to be known as the guillotine. In this country the electric chair was invented by a New York physician whose aims were similar to those of Dr. Guillotin. He wanted death to be swift and painless, although he himself was opposed to capital punishment. Even now doctors are endeavoring to find even less painful means of execution, and the direction is along poison chambers of one kind or another.

One of the most eminent of doctor-inventors was the Englishman Neil Arnott, born in 1788 at Arbroath. He received his medical education at Aberdeen and London, and after graduation became surgeon to the East India Company. After making two voyages to China, he settled down to practice medicine in 1811. In 1813 he obtained the diploma of the College of Surgeons and one year later his doctor's degree from Aberdeen. In 1855 he gave up medical practice to devote all his time to invention. From then on his contributions to practical science were of the highest order.

Among the first of his great inventions was the Arnott stove, which earned for him the Rumford Medal of the Royal Society. He also invented the Arnott ventilator and water bed. He never patented his inventions, and his rewards were mainly in medals and honors. Among the most prized of these was the gold medal at the Paris Exhibition of 1855, and the Cross of the Legion of Honor conferred on him by Napoleon III. One of his last inventions was a chair-bed to prevent seasickness. He was also one of the founders of the University of London in 1836.

The inventor of modern shorthand was a physician, Dr. Timothy Bright, born in 1551. He graduated in medicine at Cambridge in 1574. His life was barely spared two years previously while in Paris during the Massacre of St. Bartholomew's Day by his taking refuge in the home of a friend of his. He was appointed physician to St. Bartholomew's Hospital from which position he resigned in 1590. He was the first to evolve a system of shorthand writing. His system had an alphabetical basis using the initial of each word.

Two physician inventors contributed much to modern headgear. They both lived in England, and they were contemporaries. James Starton, born in 1806, was a successful skin specialist in London. His inventive genius asserted itself during his youth when he was an apprentice in a hat factory. It was he who invented the commercial process of stiffening felt hats. George Borlase Childs, born ten years after Dr. Starton, was a native of Cornwall, who upon graduation came to London where for forty years he was surgeon to the City of London Police. In 1861 he invented the modern police helmet, which is still worn by the London police.

Medical men have been prolific contributors to the science of crime detection. Among the greatest of these was Henry Faulds (1844-1930) who is credited with inventing the fingerprint method of identification. He practiced medicine after graduation, but his interest was soon focused on finding a way to identify persons. In 1880 he published his fingerprint method of identification. He issued a schedule containing outlines of the ten fingers, to be filled with imprints from them. These schedules were supplied to coroners, magistrates, surgeons, chiefs of police, etc. Faulds made the first practical contribution to the modern science of crime detection.

In the science of chemistry one of the greatest discoverers was a doctor. Friedrich Wohler graduated in medicine, but never practiced. His interests lie in chemistry to which he devoted himself with distinction. In 1836 he was appointed to the chair of chemistry at the University of Göttingen. He had had a most remarkable career before this appointment. In 1827 he discovered the metal, aluminum, one of the most useful of present-day metals.

One year later he obtained beryllium. He was the founder of modern synthetic chemistry when he produced urea synthetically for the first time.

The inventor of the modern telescopic lens was John Bevans, born 1693 who, while he practiced medicine in London, was very much interested in astronomy. He was not satisfied with the type of telescopic lens in use at that time and after a great deal of experimentation he found that by incorporating borax into the glass he obtained a lens whose refractive powers were greatly increased.

George Armstrong Peters (1859-1907), a native of Ontario, was educated at Toronto where he graduated with the degree of M.D. Several years later he became Professor of Surgery at his alma mater. Besides surgery he was much interested in horses and rifles. It was he who perfected the electric self-registering rifle target which bears his name.

Another rather versatile English doctor-inventor was Charles Brooke, born in 1804. After graduation he became a member of the surgical staff of the Metropolitan Free Hospital and Westminster Hospital. A little later he became President of the Meteorological and Royal Microscopical Societies. He was very much interested in mathematics and physics on which he lectured and wrote a great deal. He was the inventor of the self-reading barometer, thermometer and psychometer. He also invented the magnetometer which registered variations by photography. All his inventions were accepted by the observatories at Greenwich, Paris, and other centers. He also greatly improved the microscope.

A physician whose inventions have contributed very much to material progress is Sir Goldsworthy Gurney (1793-1875) who was born in Cornwall, and after graduation settled down to a surgical practice at Wadebridge. Among the greatest of his inventions was the oxyhydrogen blowpipe which is used in industry today throughout the world. The blowpipe has made possible the utilization of steel for building purposes; it is therefore responsible for all skyscrapers, for one thing.

Dr. Gurney was the first to make use of his own invention. He used the blowpipe to fuse lime and magnesia which now forms the basis of the powerful limelight, useful in photography and illumination. This is another invention of greatest value to present-day civilization. He also invented the steam jet, used in connection with locomotives, steamboats and blast furnaces as well as for the extinction of fire in burning coal mines and for cleaning and ventilating sewers. It was also Sir Goldsworthy Gurney who pointed out that a magnetic needle moves when the poles of a galvanic battery are brought into contact with the current. In his lighter moments he invented an instrument of musical glasses to be played like a piano, but this instrument's popularity lasted but for a short time.

The extramedical contributions of physicians have been of utmost value. There is scarcely a field of human endeavor which has not been invaded. The doctor as an inventor has performed and is performing a most useful service to humanity.

# CARCINOMA AND SCHISTOSOMIASIS OF THE APPENDIX\*

## A CASE REPORT

JACOB LEVINE, M.D., AND RAFAEL A. MARIN, M.D., NEW YORK, N. Y.

THIS case is presented because of the unusual association of carcinoma with schistosomiasis of the appendix.

### CASE REPORT

The patient, J. R., a twenty-eight-year-old Puerto Rican seamstress, was admitted to the Surgical Service of Dr. L. W. Crossman, City Hospital, on Sept. 21, 1933, complaining of right lower quadrant pain of several days' duration associated with nausea and vomiting. Language difficulties did not allow of any more definite history. She appeared acutely ill and showed tenderness but no rigidity in the right lower quadrant. Her pulse was 98, temperature 99° F., and respirations 24. The white blood count was 12,800 with 84 per cent polynuclear neutrophils. A diagnosis of acute appendicitis was made and an appendectomy performed four hours after admission. The postoperative diagnosis was subacute appendicitis.

*Pathologic Report.*—Gross appearance: The appendix was 11 cm. long, 6 to 8 mm. in diameter. There was a very slight bulbous swelling at the tip; externally, otherwise negative. The lumen was about normal in size, except at the tip, where it seemed to be obliterated by a solid, bright yellow, homogeneous tumor, 6 mm. in length. It apparently occupied the tissue down to muscle, except at its proximal end where it extended in the mucosa as finger-like projections. The remainder of the appendix was normal.

*Histology.*—The tumor consisted of closely packed alveoli of uniform size separated by narrow connective tissue septa. In the deeper portions, they varied more in size, were not so well delimited and showed a greater desmoplastic reaction with invasion into surrounding connective tissue and lymphatics by a few tumor cells. There was palisading of the peripheral layer of cells in the alveoli against the connective tissue septa and also against central vesicles containing a homogeneous pink-staining material. The tumor cells had a pink-gray-blue, granular cytoplasm with a smoked appearance, and, except for the palisaded cells, had indefinite cell boundaries. The nuclei were of fairly even size, ovoid to spheroid, moderately hyperchromatic, contained a few mitotic figures and occasionally formed tumor giant cells.

The tumor occupied the mucosa and widened it, compressing the outer coats. In a few regions, however, some tumor cells invaded the submucosa and even muscle. The lumen at the tip was extremely narrow and eccentrically placed. The mucosal epithelium was preserved, except in the crypts where there was replacement by tumor. Many normal gland acini were present between tumor alveoli, as were also several isolated, hyperplastic lymphoid follicles. The fibrous stroma of the tumor showed a heavy, diffuse infiltration by lymphocytes, monocytes, numerous eosinophiles, and a few plasma cells.

In the deep mucosa of the portion involved by tumor were many large tubercle-like nodules containing ova of *Schistosoma mansoni*. Many of the ova were being engulfed by giant cells. Around these nodules, in both mucosa and submucosa, was a heavy infiltration by cells similar to those mentioned above. The remainder of the appendix showed only the changes seen in a very mild, nonspecific appendicitis, and there was only a rare parasitic egg away from the tip.

\*From the Pathological Laboratory, City Hospital, Welfare Island, Department of Hospitals.

Received for publication, July 20, 1934.



*Subsequent History.*—Recovery was uneventful except for a sore throat and slight elevation of temperature seven days after the operation. She was discharged on Oct. 11, 1933, and returned a week later for further study and treatment. An inquiry in her own language revealed that as a child she was accustomed to swim in a small stream near her home

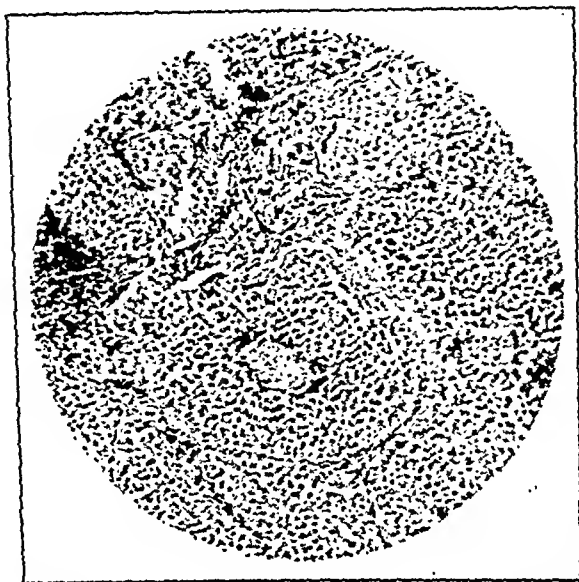


Fig. 1.—Carcinoma of appendix showing the character of the cell, the alveolar arrangement and the palisading of the basal layer.

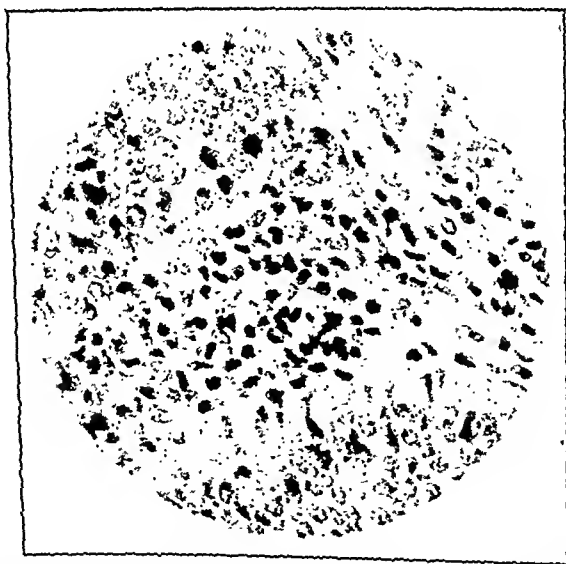


Fig. 2.—High power view of the tumor showing the type of cell, the palisading of the basal layer, the slight invasive element, and groups of mitotic figures.

(Naranjito, Puerto Rico) but that she never noticed itching or a rash following such immersions. During the past eight or ten years she had suffered occasional attacks of diarrhea, never accompanied, as far as she knew, by blood or mucus in the stools. During some periods of constipation, there was bright red blood in the feces, but this was attributed to hemorrhoids. She had never had an attack similar to that for which she was originally

admitted. A barium enema was negative. A sigmoidoscopic examination disclosed only internal hemorrhoids of moderate size. After numerous stool examinations were done, fuadin was given. This treatment caused no reaction except possibly a slight loss of appetite.

Nine different stool specimens were examined at intervals during a period of five months. From two to ten ordinary smears were examined from each specimen, making a total of 42 smears. Centrifugation and brine flotation were used on several occasions. One attempt was made to hatch ova in tap water. Ova of *Schistosoma* or the hatched miracidium could never be found. Ova of *Necator americanus* and *Trichuris trichiura*, and cysts of *Endameba histolytica* were often found. The worm ova were present in very small numbers. Trophic forms of *E. histolytica* were never encountered.

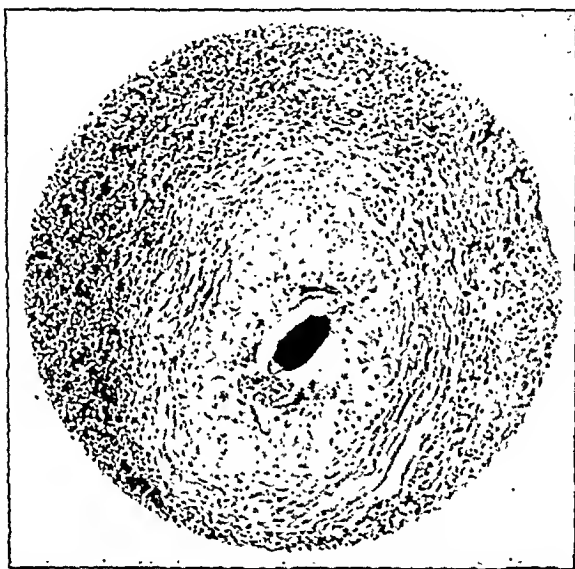


Fig. 3.—Ovum of *Schistosoma mansoni* in a tubercle-like structure and within foreign body giant cells.

#### DISCUSSION

The acute symptoms in this case are difficult to explain. The parasites other than *Schistosoma* are not held accountable for any significant degree of pathology. As regards *E. histolytica*, it has often been pointed out,<sup>1, 2, 3</sup> that in some tropical countries, in the majority of cases of infestation the parasites are carried without symptoms and without demonstrable lesions of the large bowel. Of the damage done by the *Schistosoma*, we have a good indication in the appendix. Similar pathology is likely to be present in the large bowel and in the liver. The fact that the ova could not be found in the feces suggests either that the parent worms had died at the time of examination, or, what is more likely, that the worms had strayed into parts of the body from which ova could not gain access to the feces. The prognosis depends on whether the parent worms of *Schistosoma* are still alive. If they have died or have been killed by fuadin, then, although the damage already done is irreparable, there will be no further pathology inflicted. If, however, the worms are still alive, they will lead to progressive and enlative pathology wherever they are. The neoplasm is of the type usually termed "carcinoid." It is

clinically benign and rarely metastasizes, even though histologically there is evidence of a local invasive element.

From the very close association of the carcinoma in the appendix with the presence of *Schistosoma* ova, the possibility of the tumor arising through chronic inflammation set up by the parasitic eggs must be considered.

#### SUMMARY

A case of carcinoma of the appendix associated with encapsulated eggs of *Schistosoma mansoni* is presented. The presence of the eggs practically only in that portion of the appendix having the carcinoma suggests an etiologic relationship. Ova of *Schistosoma mansoni* were never demonstrated in the stools, although three other types of intestinal parasites were found.

We wish to express our thanks to Dr. J. R. Lisa for his aid and advice in the preparation of this paper.

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### FAMILIAL RENAL GLYCOSURIA\*

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MARSHALL S. BROWN, JR., M.D., AND RUBIN POLESHUCK, A.B., NEW YORK, N. Y.

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ACCORDING to Hatlehol<sup>1</sup> the hereditary nature of the condition known as renal glycosuria was first demonstrated in 1913 by Bonniger<sup>12</sup> who showed that the condition was passed from father to son. The next year, Salomon<sup>13</sup> published three genealogic charts showing the pronounced familial tendency of this condition. Since then other writers have spoken of its hereditary nature.<sup>2, 3, 4, 5, 6</sup>

Some authors state that this condition may develop into true diabetes mellitus. Joslin has said that when cases of renal glycosuria are studied more carefully certain resemblances to diabetes mellitus may be found. However, he qualifies his statement by adding that he does not wish to be put on record as saying that one is apt to pass into the other. On the other hand, Magee<sup>7</sup> believes if a patient with renal glycosuria develops diabetes mellitus, either the former was a mistaken diagnosis, or two separate conditions exist in the same individual.

In this report we are presenting four cases of renal glycosuria occurring in three generations of one family. The history of these cases is as follows, and is also outlined in the accompanying family tree.

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\*From the Department of Medicine and Metabolism Clinics, University and Bellevue Hospital Medical College, New York University.  
Received for publication, August 14, 1931.

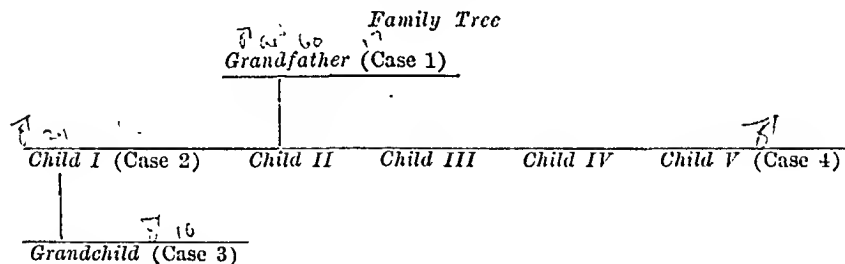
CASE 1.—The eldest of this family is an adult white male, sixty years of age, a native of Russia, who for the past seventeen years has been known to have glycosuria. This was discovered accidentally when he applied for life insurance. Neither prior to, nor subsequent to this accidental finding of glycosuria has he exhibited any of the classical signs or symptoms of diabetes mellitus. He has never been obese. Blood sugar determinations had never been done prior to the present observations.

Of his five children, all of whom are living, two have a persistent glycosuria, two have had repeatedly negative tests for glucose, both in twenty-four-hour specimens and in specimens collected two hours after meals, the fifth had sugar in his urine on one occasion four years ago. Repeated tests since then have always been negative. There are no symptoms of diabetes mellitus in any of the children. The only grandchild, Case 3, has persistent glycosuria.

CASE 2.—The eldest son of the patient in Case 1, aged thirty-nine, has had persistent glycosuria since 1917. It was first discovered accidentally at that time, when he applied for admission to the Army. No blood sugar determinations were done, and treatment for diabetes mellitus was instituted. No improvement in the glycosuria was obtained by the starvation diet then in vogue, nor later by the high fat diet. After the advent of insulin, he was treated with it, without results. Because of the absence of any symptoms, and his feeling of general good health, he discontinued further treatment. He has repeatedly been refused life insurance because of glycosuria.

CASE 3.—This boy, aged ten, son of the patient in Case 2, was discovered to have glycosuria at the age of nine, when a routine urinalysis was done during an attack of pertussis. Several specimens of urine that have been examined during the past year have all shown glucose. He exhibits no signs or symptoms of diabetes mellitus.

CASE 4.—The other son of the patient in Case 1, who has persistent glycosuria, is a fourth-year medical student, aged twenty-six, who discovered sugar in his urine during his first year in medical school, and has had glycosuria persistently since. This case has been reported by Ralli.<sup>4</sup>



The results of the glucose tolerance tests and the urinary findings are reported in Table I.

*Discussion.*—We are presenting these cases as additional evidence of the familial or hereditary nature of the condition known as renal glycosuria. These observations help substantiate the belief that it is an entirely benign condition. It is interesting to note that Cases 1 and 2 have been known to have glycosuria for seventeen and sixteen years, respectively. At its discovery the diagnosis of diabetes mellitus was made in each case and the treatment for that disease was instituted.

Case 1 shows a rise in the blood sugar level to 192.3 mg. per cent, sixty minutes after the ingestion of 100 gm. of dextrose. This, however, has returned to normal (117.6 mg. per cent) after 120 minutes. Sugar was present in the urine in definite traces in the fasting specimen, but showed an increase to a concentration of 3.7 per cent during the rise in the blood sugar. Formerly

TABLE I  
BLOOD SUGAR CURVES

		FASTING	30 MIN.	60 MIN.	120 MIN.	180 MIN.
Case 1	Blood sugar mg.	102.6	181.8	192.3	117.6	70.7
	Urinary sugar	trace	trace	3.3%	3.7%	2.3%
Case 2	Blood sugar mg.	85.1	101.0	100.0	85.8	50.00
	Urinary sugar	trace	trace	1.0%	0.59%	0.25%
Case 3	Blood sugar mg.	93.0	111.1	90.9	101.0	98.0
	Urinary sugar	---	0.5%	0.03%	0.03%	trace
Case 4	Blood sugar mg.	83.0	102.0	111.1	106.0	84.0
	Urinary sugar	Positive in all specimens.				

100 gm. of glucose was given by mouth to Cases 1, 2, and 4.  
Case 3 received 60 gm. of glucose by mouth.

with this degree of hyperglycemia a patient was considered diabetic. More recently the feeling is that a rise in the blood sugar level that is not sustained does not warrant, per se, the diagnosis of diabetes mellitus.<sup>11</sup> The history of long-continued glycosuria, the absence of any symptoms of diabetes, and the low renal threshold for glucose, together with the records of the other members of the family, establish the diagnosis of renal glycosuria in this case.

In Case 2 it is interesting, and quite significant to note that there has been no decrease in his glucose tolerance, as shown below, after sixteen years of persistent glycosuria. It is also interesting that on the basis of the diagnosis of renal glycosuria, this man has been granted life insurance of a standard rating. The diagnosis was established by the history, and glucose tolerance test, which fulfill the four cardinal requirements as given by Joslin and Magee, and the element of time, referred to by Rabinowitch and Weber, and the family history referred to by Rabinowitch.

The patients in Cases 3 and 4 have absolutely normal glucose tolerance tests.<sup>8</sup>

All of these patients showed glucose in all specimens of urine examined, even in the patient in Case 2 during the hour in which the blood sugar level range was from 85.8 to 50.0 mg. per cent.

In spite of the persistent loss of sugar in the urine there has been no loss of weight, or polyuria, in any of the four cases. In fact, the patient in Case 4 was able to gain twenty-one pounds in a six weeks' period on a high caloric diet as reported by Brown and Ralli,<sup>9</sup> and this diet in no way affected his carbohydrate tolerance. In addition, over a period of two years there has been no change in his carbohydrate tolerance as shown by glucose tolerance curves.

## SUMMARY

1. Four cases of renal glycosuria are reported.
2. The familial nature of the condition is indicated.
3. Evidence is given to show that it is a benign condition.

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# THE SIGNIFICANCE OF IRON AND COPPER IN THE BILE OF MAN\*

E. STARR JUDD, M.D., AND THOMAS J. DRY,† M.B., ROCHESTER, MINN.

WITH THE TECHNICAL ASSISTANCE OF  
MARY SUE BLEDSOE, M.S.

SINCE the classic experiments of Claude Bernard, it has been demonstrated that the liver participates in a multiplicity of physiologic processes in addition to those relating to the carbohydrates. Mention only need be made of the part that it plays in protein, fat, and mineral metabolism and in the storage of certain vitamins. Moreover, its secretions which aid digestion and absorption of food serve as a medium for the transport of excretory products as well. As a gland, the liver is unique in the sense that it possesses both secretory and excretory functions. Thus the bile salts are reabsorbed<sup>8</sup> after serving their part in digestion, whereas the bile pigments for the most part are eliminated as waste products.

Drugs such as chloroform, quinine, barbital,<sup>23</sup> and some of the halogens,<sup>17</sup> which normally do not occur in the bile of animals, have been shown to be present in the bile after their administration by mouth. The liver has even been credited with the power of excreting some of the excess urinary waste products<sup>21</sup> when the function of the kidneys becomes impaired. The diffusibility of urea, however, probably accounts for its appearance in the bile, and its rapid absorption from the upper portion of the intestines renders any increased excretion, if it does occur, unimportant so far as relieving or aiding renal function is concerned.

Varying amounts of elements concerned with the nutrition of body cells as well as those entering into the process of hemoglobin formation, in which process the function of the liver is closely related to the mineral metabolism, are present, then, at all times, in the liver. It seemed to us that a study of the bile from the standpoint of these two mineral substances most closely concerned with hemopoiesis would be a convenient starting point for the study of certain phases of the mineral metabolism, since these substances, which the liver has been shown to store, can reach the blood either directly or, possibly, through reabsorption after excretion into the gastrointestinal tract. The enterohepatic circulation of bile salts has been commented upon, and other substances in the bile may possibly share in the animal economy by being reabsorbed. Our first step was to determine whether iron and copper were present in the bile of man only under certain circumstances, or whether they were always present.

Tables I, II, and III would seem to indicate that they are constant constituents of bile. The bile of dogs and of guinea pigs has been found to con-

\*Submitted for publication July 20, 1934.

†From the Mayo Foundation.

tain from 0.09 to 0.18 mg. per cent of iron in the form of inorganic ferric compounds.<sup>23</sup>

#### MATERIAL AND METHOD

Samples of bile recovered either from the gallbladder at operation or from biliary fistulas following cholecystectomy were analyzed for their iron and copper content (Tables I and II). The method described by Kennedy was used in determinations for iron and that described by Elvehjem and Lindow for copper, with the exception that sodium pyrophosphate was utilized, as suggested by Warburg, instead of hydrogen sulphide, to remove the contained iron. The next step was an attempt to assess the respective importance of iron and copper to the remainder of the mineral metabolism of the body as well as to determine the part that the liver plays in this complex mechanism.

TABLE I  
ANALYSIS OF SPECIMENS OF BILE FOR THE CONTENT OF IRON

CASE	SOURCE OF BILE	DATE, 1932	PATHOLOGIC DIAGNOSIS	IRON, MG. PER 100 C.C.	COMMENT
1	Common duct	1/22 1/23 1/24 1/25 1/29 1/30 1/31 2/ 1	Obstruction of common duct due to stones	0.136 0.046 0.035 0.052 0.033 0.032 0.006 0.031	Biliary cirrhosis, graded 2; serum bilirubin 3.3 mg. per cent (direct); jaundice completely disappeared postoperatively; ferric citrate 40 gm. on January 27 and 28
2	Common duct	3/22 3/23 3/24	Obstruction of common duct due to stones	0.157 0.157 0.160 0.392	Suppurative cholangitis; serum bilirubin 2.6 mg. per cent (direct); liver in good condition
3	Common duct	3/22 3/23 3/24 3/30	Obstruction of common duct due to stones	0.152 0.195 0.160 0.133	Biliary cirrhosis, graded 1; serum bilirubin 8.34 mg. per cent (direct); spleen enlarged
4	Gallbladder	4/30	Cholecystitis with stones	0.0828	Liver appeared normal; no obstruction of common duct
5	Gallbladder	3/29	Chronic cholecystitis	0.175	
6	Gallbladder	4/ 8	Chronic cholecystitis with stones	0.908	No jaundice; hepatitis, graded 1
7	Gallbladder	4/ 4	Chronic cholecystitis	1.68 0.238	No jaundice; hepatitis, graded 3
8	Gallbladder	5/ 7	Cholecystitis with stones	0.115	No obstruction of common duct; no symptoms
9	Gallbladder	5/ 9	Chronic cholecystitis	0.325	No obstruction of common duct; no symptoms
10	Gallbladder	5/ 6	Chronic cholecystitis with stones	0.230	Hepatitis, graded 1
11	Gallbladder	5/ 6	Chronic cholecystitis with stones	0.146	Liver in good condition

#### IRON

Although so many details concerning the intermediate metabolic processes with which iron is concerned are as yet undetermined, these processes have nevertheless been the subject of some speculation. There is good evidence to



TABLE II  
ANALYSIS OF SPECIMENS OF BILE FOR THE CONTENT OF COPPER

CASE	SOURCE OF BILE	PATHOLOGIC DIAGNOSIS	COPPER, MG. PER 100 C.C.	COMMENT
12	Common duct	Cholangitis	0.212	Liver in good condition; dye retention, graded 0
13	Common duct	Chronic cholecystitis with stones; stone in common duct	0.063	Serum bilirubin 8.34 mg. per cent (direct); biliary cirrhosis, graded 1
14	Common duct	Stricture of common duct	0.325	Jaundice present; biliary cirrhosis
15	Gallbladder	Chronic catarrhal cholecystitis with single large stone	0.792	
16	Gallbladder	Chronic cholecystitis with multiple stones	0.158	Hepatitis, graded 2
17	Gallbladder	Chronic cholecystitis with multiple stones	0.096	Liver in good condition
18	Gallbladder	Empyema of gallbladder; multiple stones	0.094	
19	Gallbladder	Subacute cholecystitis with multiple stones	0.091	Dye retention, graded 2
20	Gallbladder	Chronic cholecystitis with stones	0.75	Hepatitis, graded 1
21	Gallbladder	Chronic cholecystitis with stones	0.349	Serum bilirubin 7.5 mg. per cent (direct)
22	Gallbladder	Chronic cholecystitis with stones	0.155	
23	Gallbladder	Chronic cholecystitis with stones	0.754	
24	Gallbladder	Chronic cholecystitis with stones	0.130	Cirrhosis, graded 1
25	Gallbladder	Chronic cholecystitis with stones	1.07	
26	Gallbladder	Chronic cholecystitis with stones; stone in common duct	0.250	Serum bilirubin 8.8 mg. per cent (direct); biliary cirrhosis, graded 2
27	Gallbladder	Chronic cholecystitis with single stone	0.906	Hepatitis, graded 1
28	Gallbladder	Chronic cholecystitis with multiple stones	0.421	
29	Gallbladder	Chronic cholecystitis with multiple stones	0.162	Serum bilirubin 4.84 mg. per cent; dye retention, graded 4; hepatitis, graded 3
30	Gallbladder	Chronic cholecystitis with multiple stones	0.880	
31	Gallbladder	Chronic cholecystitis with multiple stones	1.00	
32	Gallbladder	Chronic cholecystitis with multiple stones	0.424	Hepatitis, graded 1
33	Gallbladder	Chronic cholecystitis with multiple stones	0.723	

show, however, that iron in its more complex forms, both inorganic and organic, including the freed hemoglobin iron of physiologic and pathologic hemolysis, is not directly utilizable, and that the structural changes necessary to render it suitable for hemopoiesis occur for the most part in the liver.<sup>3</sup> The total amount of iron contained in hemoglobin is small, 0.34 mg. per 100 e.e.

TABLE III

REPORTS FROM THE LITERATURE OF CONTENT OF COPPER OF LIVER IN VARIOUS CONDITIONS

AUTHOR	PATHOLOGIC CONDITION	CASES	COPPER, MG. PER KG. OF FRESH LIVER	
			RANGE	AVERAGE
Funk and St. Clair	Hemochromatosis	1	140	140
	Normal controls	2	1 to 9	5
Herkel	Normal	12	2.88 to 12.9	7.43
	Hemochromatosis	24	10.5 to 96	22.9
	Cirrhosis	10	5.5 to 113.6	44.4
Gordon-Rabinowitch	Yellow atrophy	1	179.3	179.3
Yagi	Normal	13	6.5 to 17.5	9.58
	Normal infant	1	28.0	28.0
Flinn and von Glahn	Normal	20	2.30 to 12.42	4.84
Morrison and Nash	Normal infants	25	6.9 to 57.6	24.0
	Normal	7	1.6 to 8.5	4.0
Mallory	Normal	1	14.2	14.2
	Normal	1	0.8	0.8
	Addison's disease	1	4.6	4.6
	Alcoholic cirrhosis	1	20.0	20.0
Osterberg	Hemochromatosis	1	7.2	7.2*
Schönheimer and O'Shima	Hemochromatosis	16	9.86 to 63.29*	18.2*

\*Fixed in formalin.

of hemoglobin. Whipple and Robscheit-Robbins recently have indicated that the hemoglobin producing factor in the liver need not bear any relation to the total amount of iron in the liver. Iron is at all times stored throughout the body, depending largely on the exogenous supply; from this, "hemoglobin producing iron" may be elaborated according to the needs of the body.

The question immediately arises, therefore, as to whether this "bile iron" has been altered in such a way as to be utilizable in hemopoiesis, or whether it happens to be merely an excretion of excess iron in the body or liver. Stransky has suggested that there is a "bowel-liver-bowel circulation" of iron of much the same sort as the enterohepatic circulation of bile salts. If this is true, then the resorbed iron is probably as important in the mineral metabolism of the human body as the bile salts are in the animal economy, for with large stores of iron there should not be any necessity of resorbing the small amounts that are present in the bile. Anemia may result from the prolonged loss of bile through a biliary fistula in man as well as in dogs. In a case reported by Balderston the patient responded to treatment by bile salts, iron, and arsenic. After the level of the blood had been restored to normal, an attempt was made to maintain this by the administration of bile salts alone. This proved unsuccessful for, later, iron was again needed. The reason anemia does not occur more commonly is probably because the fistulas are very seldom complete in man. Moreover in the plasma there is a partly diffusible iron compound that Riecker<sup>19, 20</sup> and others<sup>13</sup> have shown to be decreased in anemia of the "iron deficiency" type, and to be increased in hemolytic forms of anemia in which the iron content of the liver is high and in which the administration of iron does not materially affect the course of the disease.

What further adds interest to this bile iron is the fact that, in contradistinction to many other substances investigated, it cannot be augmented to any

degree by the administration of large amounts of iron, irrespective of the route by which it is introduced into the body.<sup>23</sup>

From the foregoing facts it seems reasonable to infer that the liver acts not as an excretory organ for iron, in the sense of disposing of a waste product, but rather as a secretory organ with both qualitative and quantitative selectivity. The more does this seem so when we contrast these phenomena with the behavior of copper in the animal.

#### COPPER

The significance of copper in bile of man can only be considered with reference to all the known facts concerning its metabolism. Copper is distributed ubiquitously throughout the plant and animal kingdoms. Pharmacologically copper acts as a protoplasmic poison, but its affinity for the proteins of food and mucin of the gastrointestinal tract renders it innocuous in the amounts in which it ordinarily gains entrance to the organism. Biologically, it functions as a catalyst in formation of hemoglobin. The amount necessary for this hemopoietic function is extremely small, indeed, in sufficiently large doses copper may cause hemolysis of erythrocytes.<sup>14, 15</sup> Whether there is any relation between its catalytic activity and cell growth in general is not known, but it is of interest to note that the young, actively growing cells of plants contain copper in greater amounts than the more mature parts,<sup>16</sup> that the liver of an infant has a higher copper content than that of its mother<sup>12, 18</sup> and that copper content of the blood serum, in a variety of clinical conditions, has been found to be increased in two groups of cases, namely, in pregnant women and in advanced malignant disease.<sup>13</sup>

Experimentally, the total copper content of animals is appreciably increased by feeding the metal.<sup>2, 12</sup> In experiments in which copper is fed continuously, the absorbed copper is found in the liver almost to the exclusion of all the other viscera, irrespective of the mode of entry to the body. The amount of copper excreted in the bile steadily increases under these circumstances. With normal intake, two-thirds of the copper is excreted in the feces and one-third in the urine, but if extra copper is fed, 98 per cent of it has been removed from the feces. Flinn and Inouye recovered 1,028 mg. of copper from the feces over a period of eighty-two days when the total intake was 1,203 mg. For the same period the urinary excretion was 12.36 mg. of copper; only 11.34 mg. was recovered from the bodies, whereas 144 mg. was found to be present in the drinking tubes. Lindow and his coworkers<sup>12</sup> found that all accumulated copper is excreted in the four to five weeks following the cessation of its administration.

In specially stained microscopic sections of the liver, Mallory and Parker have demonstrated the presence of copper in the inspissated bile following its administration in large amounts to animals. Pigment gallstones have been shown to contain large amounts of copper.<sup>22</sup> The chain of events, then, is complete, and we can state with a fair degree of certainty that copper is absorbed, that what is absorbed reaches the liver, and that later it appears in the feces in amounts almost equal to those that reach the animal's tissues. Thus the liver is the most important excretory avenue for copper.

In the light of the foregoing facts the constant occurrence of copper in the bile of man may consequently be regarded as much a normal excretion of the liver as urea is a normal excretion of the kidney. The normal metabolism of copper in man is, indeed, the counterpart, in miniature, of the events depicted under experimental conditions. Dealing here not only with smaller amounts, mainly as a contaminant of food, but also with amounts that vary from time to time and from one individual to the next, we would expect to find the amounts of copper present in the liver varying with the fluctuations of exogenous supply and with the ability of the liver to excrete it.

The variations which occur in the intake of copper are well illustrated by the varying amounts of copper that are present in different foods. Lindow, Elvehjem, and Peterson determined the copper content of about 160 samples of common food material. The figures ranged from 0.1 mg. of copper per kilogram of fresh celery to 44.1 mg. per kilogram of fresh calves liver. No food examined was found to be without its copper.

When we come to consider the relation of copper to the normal physiologic conditions in the liver, the subject begins to assume a somewhat controversial form. It may be stated, however, that the bulk of both clinical and experimental evidence points to the fact that the liver can handle large amounts of copper without any demonstrable deleterious effects.

The highest figures for copper in livers in which pathologic changes are present are encountered in some cases of cirrhosis,<sup>9</sup> including hemochromatosis,<sup>6</sup> and in yellow atrophy.<sup>7</sup> Yet, in certain other cases of the former group, figures may be as low or lower than the average normal figure (Table III).

It is worthy of note that the highest figures obtained by Herkel, in his group of cases of nonpigmentary cirrhosis, are in what he described pathologically as "hypertrophic," while the exact reverse was true in those designated atrophic. It would seem that the suggestion made earlier regarding the activity of cells and their copper content may apply here, too, for the hypertrophic liver is no doubt the one that has displayed the most compensatory regeneration.

#### CONCLUSIONS

1. Copper and iron are constant constituents of the bile of man.
2. There is some evidence to suggest that iron may be excreted in the bile and resorbed from the intestines.
3. The liver is an important excretory organ for copper.
4. So far, there is no conclusive proof that copper causes injury to the hepatic parenchyma. The high figures for copper in some cirrhotic livers may be related to the degree of regeneration (cell growth) occurring within the liver.

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## AMIDOPYRINE AND GRANULOPENIA\*

### REAPPEARANCE OF GRANULOCYTOSIS IN A CASE OF RECURRING AGRANULOCYTOSIS AFTER LARGE DOSES OF AMIDOPYRINE. A CLINICAL EXPERIMENT

LOUIS R. LIMARZI, M.D., AND IVA G. MURPHY, B.S., CHICAGO, ILL.

KRACKE,<sup>1</sup> Madison and Squier,<sup>2</sup> Watkins,<sup>3</sup> Randall,<sup>4</sup> and Hoffman, Butt and Hickey<sup>5</sup> have collected and described patients in whom the onset of granulopenia was preceded by the use of amidopyrine, amidopyrine with a barbiturate, or other drugs containing the benzene ring. In six of Madison and Squier's cases who continued the use of amidopyrine drugs the mortality was 100 per cent. In two patients who recovered from the acute disease and who were later given a single dose of amidopyrine, the blood showed a rapid fall in the granulocytes. Experimentally, Kracke,<sup>6</sup> and Madison and Squier<sup>2</sup> have produced granulopenia in animals with drugs containing the benzene ring. Kracke<sup>7</sup> stressed the point that "the discussion has centered around the relationship to granulopenia of drugs containing the benzene ring and that the barbiturates have not been involved in this conception of the etiology of the disease." Exception is made in the cases described by Watkins.

On the other hand, T. H. Boughton<sup>8</sup> of Akron, Ohio, states that in the rubber factories where benzene is in general use among the workers he has seen a great many cases of benzene poisoning, yet in spite of constant watchfulness, he has found only two cases of agranulocytosis, neither of them in workers exposed to any form of industrial poisoning. Further, he states that if benzene could produce agranulocytosis, those workers who are in contact with benzene would develop the disease since these men are experiencing a subtoxic exposure. Experimentally, Reznikoff and Fullarton<sup>9</sup> came to the conclusion in their animals that benzol produces a reduction of the total white cell count and the appearance of immature granulocytes in the peripheral circulation; granulopenia was inconstant and usually an antemortem phenomenon. They suggested that "the mechanism involved in the action of benzol on the white blood cells is different from that in granulopenia." Dameshek<sup>10</sup> stressed the fact that benzene poisoning destroyed the entire bone marrow causing a severe anemia, a leucopenia, and a decrease in the number of platelets; while in agranulocytosis only the white blood cell forming tissue is attacked with an almost complete disappearance of myelocytes from the bone marrow.

The following case of agranulocytosis in which large doses of amidopyrine with codeine were given for pain was treated before the possible relationship of benzene ring drugs and granulopenia was called to our attention.

\*From the Department of Internal Medicine, University of Illinois, College of Medicine.  
Received for publication, July 25, 1934.

## SUMMARY OF PREVIOUS ADMISSIONS

Miss H. J., nurse, single, aged twenty-four years, was admitted to Research and Educational Hospital on June 17, 1930 for tonsillectomy. Physical examination was negative. No blood count was taken. Urinalysis was negative. Tonsils were removed on June 17, 1930, uneventful recovery and discharged on June 19. The only outstanding thing in postoperative course was complaint of pain in ears, which was relieved by phenol and glycerin.

*Second Admission.*—Patient was admitted to the hospital Mar. 5, 1931, acutely ill, with complaints of tightness in head and pain in left ear. Temperature was 101°, and pulse 120. Some redness in canal of left ear which was thought to be a beginning furunculosis; throat was not markedly injected. A few tender, palpable submaxillary and anterior cervical lymph nodes. Lungs presented no abnormal physical findings. Heart rate 120 with soft systolic murmur at apex. Tenderness noted over right upper quadrant of abdomen and in left lumbar region. Liver, kidneys and spleen not palpable. Reflexes normal. Past history was negative except for paracentesis of left ear drum in 1926 and an appendectomy in 1928. Blood picture: hemoglobin 11 gm.; red cells 3,970,000; white cell (average of 4 counts) 1,980; differential 46 per cent lymphocytes; 32 per cent polymorphonuclear leucocytes; 22 per cent immature cells. On March 8, white count showed 1,900 cells and only 12 polymorphonuclear leucocytes were seen on this smear (immature forms). Diarrhea developed with gross blood and mucus in the stools which were reported negative for parasites or ova. Spleen was not palpable. Temperature continued to rise and at 5:30 P.M. the patient received 500 c.c. of blood by the citrate method. She suffered a severe immediate reaction which necessitated the administration of oxygen while upon the operating table. She had a severe chill and the temperature rose to 104° later in the evening. Physical examination revealed a slightly distended abdomen and the liver was palpable for the first time. The hard palate was wrinkled and gray looking. A pink papule, 3 mm. by 3 mm. with pin point sized white excavation at the center, was noted at the superior border of the right tonsillar fossa. Bleeding points were noted along the gingival margins of the lower right side. White count was 11,500 at 10 P.M. with 10 per cent polymorphonuclear leucocytes.

Following the transfusion the patient suffered from nausea and vomiting, with an icteric tint noted in the sclerae and skin. Stools showed gross and occult blood. Urine showed occult blood 4+, albumin 2+. Four days posttransfusion there was evidence of a delayed reaction with anuria, with nitrogen retention (the nonprotein nitrogen rose to a high of 195). The urine contained 4+ albumin and casts. Fecal vomiting occurred on March 17. The blood count continued high, following the transfusion, ranging from 30,000 down to 11,500 with a differential ranging from 85 per cent to 90 per cent polymorphonuclear leucocytes, and 8 per cent to 15 per cent lymphocytes. There was a gradual lowering of the nonprotein nitrogen and general improvement in the physical condition. She left the hospital on April 6, 1931.

*Third Admission.*—Patient was admitted with complaints of chills, fever, generalized aching and severe headache on Dec. 21, 1932. Physical examination was essentially negative. There were no palpable glands. Temperature was 101°, and pulse 120. Blood picture: hemoglobin 15 gm.; red cells 5,000,000; white cells 1,720; differential count showed 22 per cent polymorphonuclear leucocytes, 60 per cent lymphocytes, 16 per cent monocytes, and 1 per cent eosinophiles. Two days later the count had increased to 2,250 with a differential showing 23 per cent polymorphonuclear leucocytes, 70 per cent lymphocytes, and 7 per cent monocytes. She was temperature-free on December 24 and was discharged.

*Fourth Admission.*—Patient was admitted on Feb. 14, 1933 with a history of pain in left ear for six months; and food disagreements. She had not felt well since previous admission in December, 1932. There was a history of ear trouble since age of seven; paracentesis in 1926 with pus draining for ten days (left ear). She had had aching pain over the left mastoid area. Roentgen plates showed normal left mastoid. Blood picture on admission: hemoglobin 14 gm.; red count 4,900,000; white count 4,100; differential showed 30 per cent polymorphonuclear leucocytes, 45 per cent lymphocytes, 19 per cent monocytes, 3 per cent eosinophiles, and unclassified 1 per cent. Highest temperature was 99.8°. Discharged on Feb. 18, 1933.

TABLE I  
FIFTH ADMISSION

DATE	TOTAL COUNT			DIFFERENTIAL COUNT IN NUMBERS						MEDICATION
	HB.	RED	WHITE	P	L	M	E	B	U	
7/10/33	9.5	4,110	3,550		25					Codaine gr. 1, pyramidon gr. 10.
7/11/33	.			8	79	13				Codaine gr. 2, pyramidon gr. 20.
7/12/33			2,850	1	79	13				Yellow bone marrow gr. 60 t. i. d., liver ext. gr. 30 b. i. d., 2.5 c.c. intramuscularly. Aspirin gr. 15, pyramidon gr. 50, codeine gr. 1.
7/13/33			2,850	1	52	4				Pyramidon gr. 40, aspirin gr. 5, luminal gr. 1/2, phenacetin gr. 10, codeine gr. 1, amytal gr. 3, bone marrow gr. 60 t. i. d., liver ext. 1/2 oz. t. i. d.
7/14/33			2,850	1	52	4				Aspirin gr. 5, codeine gr. 3, pyramidon gr. 40, bone marrow gr. 60 t. i. d., liver ext. 1/2 oz. t. i. d.
7/15/33			2,350	5	88	2				Codaine gr. 1 1/2, pyramidon gr. 40, pantopon gr. 1/6, liver ext. 1/2 oz. t. i. d., bone marrow gr. 60 t. i. d., liver ext. 3 c.c. intramuscularly.
7/16/33			2,450	21	67	5			6	Codaine gr. 3, pyramidon gr. 30, pantopon gr. 1/2 (li), bone marrow gr. 60 t. i. d., liver ext. 1 1/2 oz. t. i. d., pantopon gr. 1/3 (o).
7/17/33			1,700	12	35		1		3	Codaine gr. 2, aspirin gr. 25, pyramidon gr. 60, haliver 20 drops, belladonna 8 drops b. i. d., liver ext. 1/2 oz. t. i. d., bone marrow gr. 120 t. i. d., sodium bromide gr. 45 (retention enema), morphine sulphate gr. 1/6.
7/18/33			2,800	71	29					10% glucose intravenously (total 2,000 c.c.), morphine sulphate gr. 1/6, codeine gr. 2, pyramidon gr. 10, bone marrow gr. 120 t. i. d., liver ext. 1/2 oz. t. i. d., belladonna 8 drops b. i. d.



TABLE I—CONT'D

DATE	TOTAL COUNT			DIFFERENTIAL COUNT IN NUMBERS						MEDICATION
	Hb.	RED	WHITE	P	L	M	E	B	U	
7/19/33			2,800	61	36	2				Codeine gr. 3, pyramidon gr. 20, bone marrow gr. 60 t. i. d., liver ext. ½ oz., belladonna 8 drops b. i. d., liver ext. 3 c.c. intramuseularly.
7/20/33			2,750	60	35	5				Morphino sulphate gr. ⅙, codeine gr. 1, pyramidon gr. 10, bone marrow gr. 60 t. i. d., liver ext. ½ oz. t. i. d.
7/21/33			4,150	67	32	1				Venoclysis 1,000 c.c. 5% glucose, morphino sulphate gr. ⅙, bone marrow gr. 60 t. i. d., liver ext. ½ oz. t. i. d., belladonna 8 drops b. i. d.
7/22/33			5,200	74	20	4			2	Codeine gr. 1, pyramidon gr. 20, bone marrow gr. 60 t. i. d., liver ext. ½ oz. t. i. d., belladonna 8 drops b. i. d.
7/23/33										Bone marrow gr. 60 t. i. d., liver ext. ½ oz. t. i. d., belladonna 8 drops b. i. d.
7/24/33			6,675 (av.)	69	30	1				Pyramidon gr. 10, bone marrow gr. 60 t. i. d., liver ext. ½ oz. t. i. d., belladonna 8 drops b. i. d.
7/25/33			6,650	68	30	2				Liver ext. 3 c.c. intramuseularly.
7/26/33			5,700	62	35	3				
7/27/33			8,650	71	25	3				Total pyramidon (amidopyrine) gr. 370

P, Polymorphonuclear leucocytes.

L, Lymphocytes.

M, Monocytes.

E, Eosinophiles.

B, Basophiles.

U, Unclassified.

*Fifth Admission.*—Patient was admitted July 10, 1933 complaining of soreness in upper jaw, sore throat, aching in bones and muscles, and intermittent earache. She stated that she had been quite well until July 6, 1933 when she began to have soreness in the upper jaw, apparently in the teeth and an associated bilateral earache which was severe enough to prevent her from sleeping. Sore throat appeared on July 8, 1933, yet she continued on floor duty. On July 9, after a sleepless night during which her temperature was 101°, she vomited and was unable to work. Generalized body pain appeared on July 10. Physical examination revealed a young woman, in a good state of nutrition, who was tossing restlessly in bed, apparently quite ill. No mastoid tenderness was noted. Gums were coated at tooth margins with a white friable material which was easily removed. Pharynx was glazed and injected with irregular granules scattered throughout the posterior pharyngeal wall. Submaxillary, sublingual, and anterior cervical lymph nodes were palpable. Axillary glands were enlarged and tender. Lungs showed normal expansion; a few scattered râles were heard posteriorly near the angles of scapulas. Heart borders were within normal limits. A systolic murmur was present at the apex. The liver, spleen and kidneys were not palpable. Temperature was 101°; hemoglobin 9.5 gm.; red cells, 4,110,000; white cells 3,550. Smear showed 25 lymphocytes (see Table I).

The patient was placed on yellow bone marrow, 60 gr. t. i. d., liver extract orally and intramuscularly, aspirin and pyramidon, or codeine and pyramidon for relief of pain (see Table I). Her general condition remained unchanged. The white count dropped to 2,850, the smear showing 1 per cent polymorphonuclear leucocytes, 79 per cent lymphocytes, and 13 per cent monocytes. The aching became more generalized and on July 15 her condition was not improved. She complained of pain in both ears. Otoscopic examination revealed no pathology. The gums still showed white friable material. There was a grayish sloughing area in the left lower jaw on the inner side. The white count was 2,350 with 5 per cent polymorphonuclear leucocytes, 88 per cent lymphocytes, and 2 per cent monocytes. The count did not materially increase in total white cells but polymorphonuclear leucocytes appeared, 21 per cent, and lymphocytes dropped to 67 per cent. During this time pyramidon was included in the medication in amounts varying from 20 to 50 gr. daily. A marked swelling appeared on the left side of the face accompanied by severe pain and the total count dropped to 1,700 showing a total percentage of 24 for polymorphonuclear leucocytes and 70 for lymphocytes. Only 51 cells could be counted on the smear. Intravenous glucose was instituted on July 18; swallowing was very painful due to the sloughing area in the jaw, which later extended down into the pharynx. The total count began to rise with the appearance of the slough. At a count of 2,800, the polymorphonuclear leucocytes constituted 71 per cent, lymphocytes 29 per cent of the blood picture. Nucleotide was withheld, owing to the improvement in the blood picture.

The improvement was continuous; the necrotic areas in the pharynx disappeared; the total count was low at first but began to rise, while polymorphonuclear leucocytes and lymphocytes occupied their normal proportions in the differential counts. She was discharged on July 27, 1933 with the advice to continue the yellow bone marrow and liver extract.

#### COMMENT

That this is a case of recurring granulopenia and not one of aleucemic lymphadenosis or aplastic anemia is shown by the selective disappearance of the granulocytes. The lymphocytes were always present in normal or increased numbers. In the lowest white count on July 17, 1933, of 1,700 there were 1,166 lymphocytes which is but little below the normal lymphocyte level. Most of the counts when analyzed show an actual increase in lymphocytes, as on July 15, 1933, when the total white count was 2,350 and the number of lymphocytes was 2,188. No abnormal forms of lymphocytes were seen on any of the smears. The hemoglobin was moderately reduced but the red blood count was normal throughout.

The only treatment the patient received during her first attack in 1931 was a 500 c.c. blood transfusion and small amounts of aspirin and pyramidon for pain. There is no doubt that the severe reaction (elevation of nonprotein nitrogen, etc.) was directly due to the transfusion. In the second attack (third admission) the patient showed a leucopenia with a moderate granulopenia, generalized pains, and temperature elevation. During her fourth admission in 1933 (Feb. 14 to Feb. 18), only one blood count is recorded, which, however, showed that she was in a neutropenic phase at this time.

In her severe attack in 1933 (fifth admission) the complete picture of agranulocytosis (fever, generalized pains, ulceration of mouth, leucopenia, granulopenia) manifested itself. The total white count was 2,550 on admission. On examination of one smear no granulocytes were found and only 25 lymphocytes. The following day the differential showed 8 per cent neutrophils. A few of these were immature forms. From July 12 to July 14, 1933, there was a persistent leucopenia and neutropenia (only one neutrophile was seen on the blood smears). On July 15, 1933, and the days that followed, despite the progressive leucopenia, there was a gradual increase in the percentage of neutrophils. On July 17, 1933, the day of the lowest total white count recorded (1,700), the differential smear showed 12 neutrophils out of a total of 51 cells counted, or 24 per cent. Beginning on July 18, 1933, and until her discharge from the hospital on July 27, 1933, there was a gradual increase in the total white count and a progressive increase in the percentage of polymorphonuclear leucocytes. She left the hospital with a normal blood picture. The treatment consisted of yellow bone marrow by mouth, liver extract orally and parenterally. During the few days in which swallowing was difficult, 5 per cent glucose in buffer solution was administered by vein.

This nurse states that she has taken 15 to 20 gr. of amidopyrine, with or without aspirin, each week for the last four years, and in the fourth attack (fifth admission), which is summarized in Table I, it will be noted that the patient received a total of 370 gr. of amidopyrine. It should be further noted that the granulocytes reappeared, increased in number, and raised the total leucocyte count in spite of the daily administration of this drug. Since her recovery the patient has been given amidopyrine in 15 gr. doses without producing any change in her white count or differential. The patch test with amidopyrine on the arm was negative.

Kracker and Parker<sup>11</sup> state that "in the clinical or experimental development of granulopenia it is necessary to presuppose the existence of a previously weakened, damaged or idiosyncratic bone marrow which may be congenital or acquired." In this patient if we assume that an inferior or weakened bone marrow is present, these large doses of amidopyrine did not depress granulocytosis.

The question naturally arises as to whether the amidopyrine is to be regarded as an agent showing general toxicity to bone marrow or one to which a patient may become specifically susceptible and from which a selective toxic action on the bone marrow may arise. The failure of the drug to depress granulocytosis in this case would point to the latter view.

## SUMMARY

1. A case of recurring granulopenia is reported in which there were recoveries from four attacks. In all four there was a granulopenia and in three a leucopenia.

2. An analysis of the differential blood counts shows that the case was one of granulopenia and differentiates it from an aleucemic lymphadenosis and an aplastic anemia.

3. Recovery from the first attack followed a blood transfusion which was associated with a severe immediate and delayed reaction. Recovery from the second and third attacks occurred spontaneously. Recovery from the fourth attack occurred after the administration of yellow bone marrow and liver extract (by mouth and parenterally).

4. In the fourth attack large doses of amidopyrine (a total of 370 gr.) were administered during the malignant phase and were continued into the recovery phase, showing that it was not possible to depress the granulocytosis by the administration of doses of amidopyrine of this size.

5. Since recovery from the last attack, 15 gr. doses of amidopyrine have been given without influencing the total or differential white count.

6. The amidopyrine patch test was negative.

We wish to thank Dr. Robert W. Keeton, at whose suggestion this report has been prepared, for the privilege of reporting this case.

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# ACTION POTENTIALS FROM INTERCOSTAL MUSCLES BEFORE AND AFTER UNILATERAL PNEUMECTOMY\*

FREDERICK M. ANDERSON, M.D., AND DONALD B. LINDSLEY,† PH.D.,  
BOSTON, MASS.

THE importance of afferent impulses from the lungs by way of the vagus nerves has been stressed since the investigations of Hering and Breuer<sup>1</sup> and more recently the work of Partridge,<sup>2</sup> Adrian,<sup>3</sup> and Hammouda and Wilson<sup>4</sup> has borne this out. Barry<sup>5</sup> emphasized the part played by impulses of visceral origin via the sympathetic chains and also those arising in the thoracic walls and traveling over the somatic nerves. The influence of these impulses on respiratory movements has been extensively investigated by these and other authors, but no one seems to have attempted to differentiate between the two sides of the thorax when impulses from one side only are interrupted.

In two patients at the Massachusetts General Hospital, following total unilateral pneumectomy for carcinoma of the lung, a persistent, almost complete lack of respiratory movement on the operated side was noted. This suggested the possible preponderance of a unilateral as opposed to a bilateral influence of the pulmonary afferent fibers on the respiratory center and indirectly on the extrinsic muscles of respiration. It was decided to study the motor innervation of the two sides of the thorax separately in normal cats and again in cats subjected to total unilateral pneumectomy, thus duplicating the condition of the patients except for the complicating neoplasms, adhesions, infection, and stiffening of the mediastinum in the latter.

## METHOD

Cats anesthetized with sodium barbital (0.040-0.045 gm. per kilo in 10 c.c. of normal saline injected intraperitoneally) were prepared for the recording of action potentials from the external and internal intercostal muscles of the thoracic wall. The cats were placed in a supine position and all limbs were fastened securely so that the original position of the animal was maintained throughout. Flaps comprising the layers of skin and muscle down to the slips of origin of the serratus anterior and external oblique were turned back by sharp dissection. The slips of serratus anterior and external oblique were left in place to protect the intercostal muscles from exposure but were underent to allow accurate placing of the needle electrodes.

The pneumectomies were performed aseptically using intratracheal ether and an automatic respiration pump. An incision was made through the eighth intercostal space and the lung removed at the hilus with separate ligation of the pulmonary artery, vein and bronchus, plus a mass ligation. Silk was used

\*From the Surgical Laboratories of the Harvard Medical School at the Massachusetts General Hospital and the Laboratories of Physiology in the Harvard Medical School.  
Received for publication July, 31, 1931.

†National Research Fellow.

throughout and the operated side was aspirated following closure of the incision. Recovery was uneventful, and when examined after death the hilus was healed over smoothly, and there was no sign of infection or adhesion between the mediastinum and the thoracic wall. The later investigations were carried out four weeks postoperatively.

For studying the motor innervation of the intercostal muscles we have made use of the single motor unit technique which permits the recording of action potentials from a group of muscle fibers innervated by a single motor neurone. This technique was first successfully used by Adrian and Bronk<sup>4</sup> and has been described more fully by Adrian.<sup>7</sup> The nature of the motor unit has been discussed very thoroughly by Eccles and Sherrington<sup>8</sup> and Sherrington.<sup>9</sup>

The electrodes used in this investigation for isolating the response of single motor units were of the coaxial type. They consisted of a hollow steel hypodermic needle which served as a ground lead and a fine insulated wire cemented into its lumen serving as a grid lead to the amplifier.

The action potentials were led off to a six-stage, transformer-coupled amplifier\* which drove either a loud speaker for listening to the responses or a Du Bois oscillograph for recording them. The electrodes were inserted in the muscle mass of one of the thoracic interspaces in such a manner that the shielded side of the hypodermic needle tended to reduce the interference by spread of electric currents from the heart. Even though such precautions were taken the electric response of the heart, which is a frequent intruder in the region of the thorax, appears in some of the records.

When the responses of one or more motor units were heard clearly in the loud speaker during a respiratory cycle the output of the amplifier was switched to the oscillograph and a record obtained. The reflection of a beam of light from the oscillograph mirror was photographed upon moving bromide paper by means of an electrocardiograph camera.

## RESULTS

An attempt was made to judge the degree of activation of the external intercostal muscles by two methods, one, the number of activated units found on inserting the electrodes into the muscles of each side at random, two, the frequency of the response in single motor units from as nearly corresponding locations on the two sides as possible. The external intercostal muscles of the third to the seventh interspaces were used for this sampling.

*Normal Cats.*—When the coaxial needle electrodes were inserted into the external intercostal muscles of the two sides in normal cats no consistent difference in the degree of activation could be found.

The frequency of the response of nearly all the individual motor units varied within the range of 6 to 14 per second, with most units responding at a frequency of from 8 to 10 per second. Fig. 1 shows a typical record. The average frequency of discharge of motor units on the two sides was found to

\*A portable amplifier designed and constructed by E. L. Garceau of the Harvard Medical School Electrical Laboratory.

be nearly equal. To obtain these averages, the responses from at least two motor units in each of three interspaces on each side were recorded during two respiratory cycles.

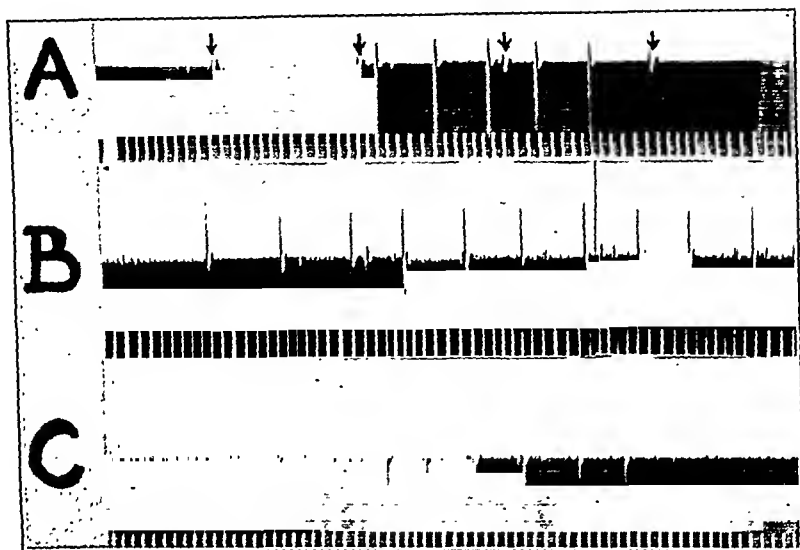


Fig. 1.—A and B, typical action potential records from single motor units of the external intercostal muscles of the cat during inspiration. In A the intruding electrocardiogram is indicated by arrow. C, record of individual motor unit potentials from internal intercostal muscles during expiration. Time at bottom of record in 1/50 second intervals.

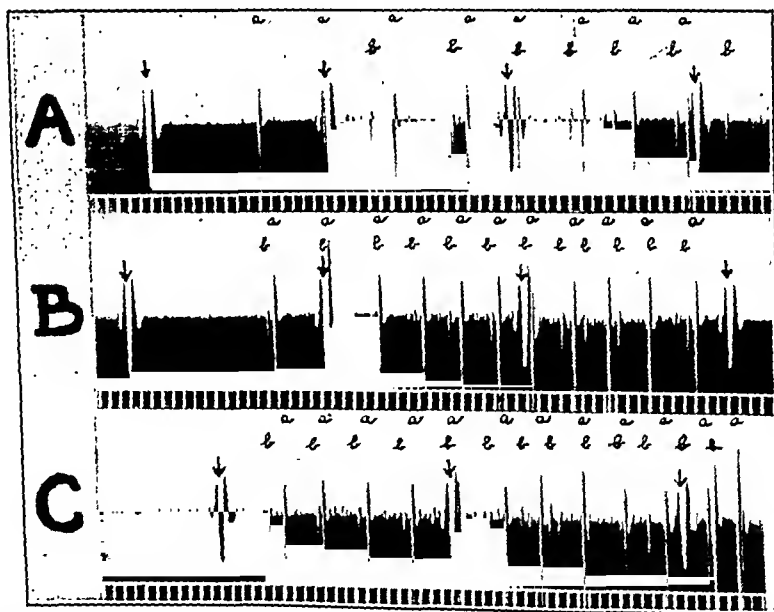


Fig. 2.—A, action potentials from two motor units (a and b) of the external intercostal muscles during normal inspiration. B and C, same units after ten and thirty seconds, respectively, of carbon dioxide inhalation, showing increased frequency of motor unit responses. The electrocardiogram is indicated in all records by arrow. Time in 1/50 second intervals.

In order to determine the range of frequency through which any individual motor unit might vary, hyperpnea was produced by administering carbon dioxide. Contact was maintained with the same motor unit previous to and during the administration of carbon dioxide. It will be noted in Fig. 2 that the frequencies of the motor unit responses were normally 9.1 and 9.6 per second, respectively. After ten seconds of carbon dioxide inhalation each increased to 13 per second and after thirty seconds they were 14.9 and 14.6 per second. A similar response to inhalation of carbon dioxide was noted with other motor units.

In all preparations the internal intercostal motor units were sampled as well. We consistently found action potentials in these muscles during expiration (see Figs. 1-C and 3), thus confirming Adrian's<sup>3</sup> finding that expiration is at least in part an active process. Although a rhythmic expiratory grouping of discharges was the usual finding from internal intercostal motor units, occasionally units discharging continuously through all phases of the respiratory cycle were noted.

*Effects of Unilateral Pneumectomy.*—One month after operation studies identical with those on the normal control cats were made. No differences of gross activation on the two sides could be detected since motor unit responses

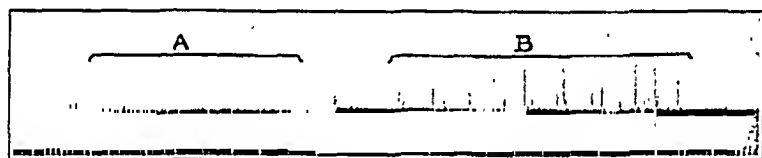


Fig. 3.—Action potentials during inspiratory and expiratory discharges, with electrodes recording from both external and internal intercostal muscles. A, single motor unit response from internal intercostal muscle during expiration. B, responses of two individual units from external intercostal muscle during inspiration. (Inspiratory and expiratory movements of chest wall were observed and correlated with their respective audible motor unit responses.) Time in 1/50 second intervals.

could be obtained equally well from either side. The average frequencies of response of the individual motor units on the two sides were also found to check as closely as did those in the control cats.

In those records obtained from the operated side a more marked tendency toward synchronization of motor unit responses was found. This synchronizing tendency was noticed in action potentials which by ear seemed to be from single motor units but which were shown photographically to be made up of several units activated almost simultaneously.

#### DISCUSSION

Clinical observation of patients subjected to total unilateral pneumectomy indicated a possibility that the withdrawal of the afferent impulses from one lung might influence the movements of the ipsilateral extrinsic muscles of respiration to a greater degree than the contralateral.

Since it is generally accepted that the contraction of a muscle may be graded in two ways, i.e., by variation in the number of motor units brought into action



and by changes in the frequency of the impulses activating each individual motor unit, we have used these two criteria as a measure of the degree of activity of the two sides.

In both normal and unilaterally pneumectomized cats the number of activated intercostal motor units on each side of the thorax appeared to be of a similar order. This was gauged entirely by ear while plunging the needle electrodes into the muscle and is only roughly quantitative.

The average frequency of discharge in the individual motor units was also determined. These averages were found to vary in both the normal and operated animals from 6 to 14 per second, but in each animal a rather close correspondence was found between the two sides.

It is, therefore, concluded that interrupting the afferent impulses from one lung (i.e. unilateral pneumectomy) does not produce any gross difference between the intercostal activation of the two sides. Small differences may exist which our method is not able to detect, for the variation in frequency of the motor unit response is of such an order that many more units than were used would be required to obtain an accurate average. A further source of error might be introduced through conscious or unconscious selection of motor units of a standard frequency or duration. This we have attempted to avoid.

A notation was made above concerning a tendency for synchronization of action potentials on the operated side. If the respiratory center were possessed of the power for spontaneously emitting rhythmic efferent discharges, as is strongly evidenced by the work of Adrian and Buytendijk<sup>10</sup> on the brain stem of the goldfish, it might well be supposed that a considerable degree of motor cell synchronization would exist. In the intact animal the play of afferent impulses, especially of those from the lung, impinging on this center or on neurones below it would tend to break up this synchronization by influencing the different cells to varying degrees. It is possible that, by removing many afferent stimuli from one side the impulses initiated by the cells of the respiratory center have come nearer to reaching the muscles in their more natural rhythmic form. In agreement with this is the work of Dusser de Barenne and Brevée<sup>11</sup> on the *triceps brachii* and other skeletal muscles which showed a tendency toward greater regularity of the electromyogram after local narcosis of the sensory spinal mechanism had cut off centripetal impulses.

#### SUMMARY

1. It has been demonstrated that the external intercostal muscles of the two sides of the thorax in normal cats are activated equally, both in regard to the number of units involved and the average frequency of the responses in each.

2. Similar studies of the two sides of the thorax in cats subjected to total unilateral pneumectomy have shown that there is no demonstrable difference between the two sides in the number of activated units and in their average frequencies of response.

3. A tendency toward synchronization of motor unit responses on the operated side was noted.

4. Action potentials recorded from motor units of the internal intercostal muscles during expiration indicated that expiration is at least in part an active

process. Responses continuing throughout the respiratory cycle were recorded occasionally from the internal intercostal motor units.

The authors wish to express their thanks to Dr. E. D. Churchill, whose clinical observation suggested this problem, and to both him and Dr. Hallowell Davis for making laboratory facilities available.

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# LABORATORY METHODS

## THE COMPLEMENT FIXATION IN THE DIAGNOSIS OF LYMPHOGRANULOMATOSIS VENEREA\*

WALDEMAR E. COUTTS, M.D., AND TEGUALDA PONCE, M.B., SANTIAGO, CHILE

EVER increasing frequency of different syndromes due to lymphogranulomatos venerea, the general nature of the disease and the interest of establishing a more practical method of diagnosis than the testing of cutaneous allergy, induced us to study the complement fixation test in all clinical conditions attributed, up to the present moment, to this illness.

Our starting point was the preparation of a specific antigen. Preliminary studies to this end, reported and published last year in *Medicina Moderna and Dermatologische Wochenschrift*, had allowed us to establish that aqueous antigens were to be preferred.

After a period of over seven months' experience we have arrived at the conclusion that the most reliable antigen is one prepared as follows:

1. Surgically excised ganglia are finely cut and minced in a sterile mortar reducing them to pulp. The ganglia must be from a patient with negative Wassermann, Kahn, Mantoux, and Ito von Reenstierna reactions. They must not have broken out spontaneously or been previously punctured.

2. Pulp is mixed per gram with 10 c.c. of a 0.5 per cent carbolic acid solution. Mixture is well shaken for half an hour and next centrifuged for from ten to twenty minutes.

3. Supernatant liquid is drawn off by means of a pipette and placed in a water-bath for one hour at 56° C.

Titration of the antigen is made in the presence of highly positive lymphogranulomatous sera and following the usual standard practices.

For performing the reaction we use a small amount of serum, accurate titration of the hemolytic system and simplification of the distribution. The decrease in the amount of serum as noted in the explanatory chart, does not diminish the sensitiveness of the reaction, provided the proper doses of antigen and complement are used.

	CONTROL	R. I	R. II	R. III
Serum	0.3	0.3	0.3	0.3
Complement	0.2	0.2	0.2	0.4
N/Salt Solution	1.3	0.8	0.8	0.6
Antigen		0.4	0.5	0.6
Hemolysis	0.5	0.5	0.5	0.5
Red B.C.	0.5	0.5	0.5	0.5

\*From the Laboratory of the Department for Social Hygiene, Chile Public Health Service. Received for publication, May 17, 1934.

Owing to the frequency of positive syphilis reactions in patients suffering from lymphogranulomatous syndromes, we have once and again tested the antigen in presence of highly positive syphilitic sera. In over one hundred controls we have never registered a positive reaction, employing dilutions of  $\frac{1}{50}$ ,  $\frac{1}{100}$ ,  $\frac{1}{200}$ , and  $\frac{1}{250}$  of lymphogranulomatous antigen. We have also submitted sera of normal children to the test and registered analogous results. Up to the present moment we have performed over 300 lymph reactions at the request of different specialists. Personally we have been able to follow its course in over 30 patients. The survey of data in our possession which we condense in Table I will serve to illustrate our conclusions.

TABLE I  
LYMPH REACTION (L. R.)

NAME	AGE YEARS	SYNDROME	EVOLUTION	L. R.	W. R.	K. R.
A. M.	30	Ing. lymphogr.	93 days	+	-	-
J. M.	33	Ing. lymphogr.	150 days	+	-	-
R. P.	29	Ing. lymphogr.	45 days	+	++	+++
G. B.	24	Ing. lymphogr.	20 days	+	-	-
J. V.	35	Ing. lymphogr.	65 days	+	-	-
M. N.	24	Ing. lymphogr.	8 days	-	-	-
J. C.	22	Ing. lymphogr.	20 days	-	-	-
P. L.	23	Ing. lymphogr.	12 days	-	-	-
E. P.	36	Ing. lymphogr.	10 days	-	-	-
A. C.	28	Ing. lymphogr.	15 days	-	+	++
S. V.	26	Esthiomena	150 days	++	-	-
M. C.	48	Eleph. pen. and se.	4 years	+++	-	-
L. I.	22	Esthiomena	5 years	+	+++	+++
P. N.	40	Gen. ano. rectal	4 years	+	-	-
C. G.	24	Ing. lymphogr.	30 years	+	-	-
C. G.	24	Ing. lymphogr.	57 years	+	-	-
A. C.	26	Ing. lymphogr.	15 years	-	-	-
A. C.	26	Ing. lymphogr.	38 years	+	-	-
A. C.	26	Ing. lymphogr.	71 years	+	-	-
J. S.	19	Ing. lymphogr.	30 years	+	-	-
J. S.	19	Ing. lymphogr.	90 years	+	-	-
J. S.	19	Ing. lymphogr.	106 years	+	-	-
E. I.	22	Ing. lymphogr.	20 years	-	+	++
E. I.	22	Ing. lymphogr.	26 years	+	-	-
L. M.	25	Ing. lymphogr.	13 years	-	-	-
L. M.	25	Ing. lymphogr.	25 years	+	-	-

#### CONCLUSIONS

1. Lymph reaction is not prematurely positive; from twenty to twenty-five days after the onset of symptoms, there appear the first signs of positivity.
2. While the process is inactivity the reaction persists positive.
3. We believe it to be specific for the diagnosis of lymphogranulomatous syndromes.

# TECHNIC OF URINALYSIS IN FAT EMBOLISM\*

## CLINICAL AND EXPERIMENTAL STUDY

FRANK J. JIRKA, M.D., AND CARLO S. SCUDERI, M.D., CHICAGO, ILL.

THE authors have been studying the problem of "fat embolism in fractures" for a number of years. This study has necessitated the examination of several hundred urine specimens for the presence of fat in the urine of suspected cases. After a period of time, it was found that this apparently

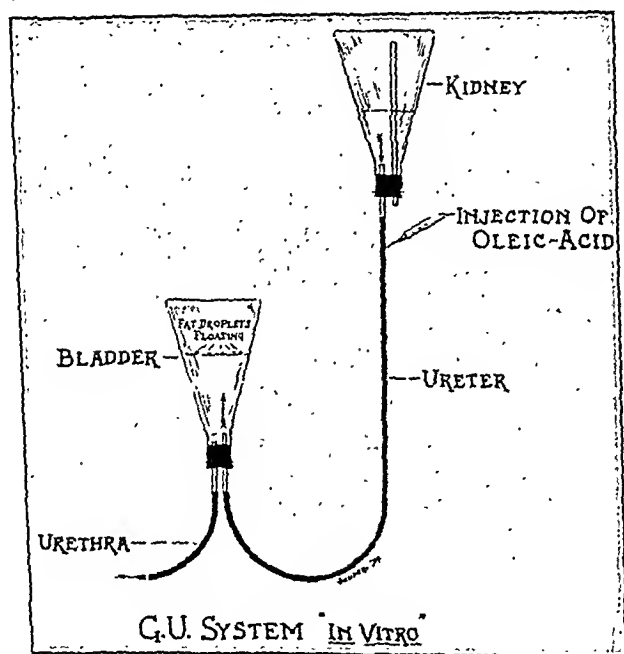


FIG. 1.

simple investigative phase of the problem offered an interesting physical phenomenon which heretofore has evidently not been sufficiently emphasized, and for this reason was investigated by us, at this time.

That fat is excreted by the kidneys into the urine of patients suffering from fat embolism, is well known and often spoken of in the literature. But unfortunately its detection is difficult because of one very elementary physical phenomenon; the fat floats on the surface of the urine, and therefore is not excreted from the bladder unless the last few cubic centimeters of urine are

\*From the Surgical Service of Cook County Hospital.  
Received for publication, July 21, 1934.

expressed. For this reason many urines examined which contain fat, appear negative because of this simple oversight.

To prove conclusively this contention, two flasks were used to carry this experiment *in vitro*. The set-up used was as illustrated in Fig. 1. The flask above represented the kidney, the one below, the bladder. These were connected by a rubber tube representing the ureter. A second rubber tube from the lower flask represented the urethra. The fluid was permitted to run very slowly from the top flask to the lower, and fine droplets of oleic acid were injected into the tube close to the top flask, with a hypodermic syringe. In this manner we had a visual genitourinary system.

As one looked into the lower flask, it was noticed that as the fluid accumulated in the lower flask, the fat droplets rose to the top. (In both the *in vitro* and clinical experiments, 2 c.c. of oleic acid were used, divided into small droplets by simple shaking with water.)

After the lower flask was filled, the pinch clamp on the rubber tube representing the urethra was removed, and the fluid permitted to flow out. The

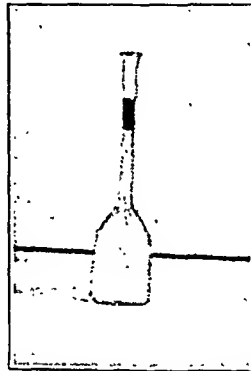


Fig. 2.—Illustrating a Babcock tube as used for butter fat determinations. The uppermost dark layer is the centrifuged fat, which can be measured on the graduations.

entire contents of the flask, with the exception of the last few cubic centimeters of fluid, were absolutely fat free. (The authors have used both urine and plain tap water as the fluid in the *in vitro* experiment, and found the physical phenomena the same.)

In order to prove conclusively what we saw so well demonstrated *in vitro*, the experiment was carried to the human being. With the kind cooperation of Dr. P. Nelson of the Urology Department, the pelvis of the kidney was injected with 2 c.c. of sterile oleic acid, in cases following retrograde pyelography. Four cases were tried, two males, and two females.

Two to three hours after the fat injection into the pelvis of the kidney, the males were instructed to pass the urine into five to seven flasks, excreting not more than two fingers depth in each. In both cases, the last flask contained all of the oleic acid, while the others were absolutely fat free.

Due to the difficulty of collecting the divided urine specimens in the female, these subjects were catheterized with glass catheters. In both cases the urine flow ceased without evidence of any fat floating in the flasks, but

when the patient was compelled to sit up, the oleic acid filled the glass catheter, and not another drop of urine was obtained.

In each case the last portion of urine with the fat was placed in a Babcock tube, as used for butter fat determination in milk, and centrifuged. Less than 0.1 of 1 c.c. was found to be lost in the manipulation of each specimen.

#### CONCLUSIONS

1. Unless the bladder is completely emptied in the sitting or erect posture, examination of the urine for fat is worthless.
2. All of the intracystic fat will be found in the last few drops of urine, due to the physical phenomenon of floatation of fats and oils.
3. Catheterization of the female is imperative; in the male it is more reliable than voluntary micturition.
4. Direct quantitative readings of the amount of fat present in the urine can be easily obtained by placing the specimen in a Babcock tube, and centrifuging for fifteen minutes.

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### A SIMPLE AND ACCURATE METHOD FOR STANDARDIZING THE CELL VOLUME PERCENTAGE OF ANY BLOOD FOR SEDIMENTATION TEST\*

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HUEI-LAN CHUNG, M.D., PEIPING, CHINA

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A PERUSAL of the literature reveals that there is as yet no standardized method of performing the blood sedimentation test. The anticoagulants used vary from inorganic salts to biologic products, and these again vary from powders to solutions of different concentrations. The sedimentation tubes employed vary greatly in their size, height, and shape. The length of these tubes varies from about 60 mm. to more than 300 mm., their internal diameter from 1 mm. or less to 11.2 mm., their shape from cylindrical to rectangular, and their bottom from flat to conical. Furthermore, the methods of recording the rate of sedimentation also differ widely. The three principal methods for determination of the sedimentation rate today are: (1) the *time method* of Linzenmeier<sup>1</sup> in which the time required for the blood cells to fall a certain distance is measured; (2) *distance method* of Fåhræus<sup>2</sup> and Westergren<sup>3</sup> in which the distance the blood cells fall in a given length of time is measured; and (3) the *graphic method* of Cutler<sup>4</sup> in which the distance sedimentation of blood cells is measured at frequent interval. The period of time for construction of sedimentation curves. Again methods has many modifications. It is evident, therefore, formed with such widely divergent technics will give results

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\*From the Department of Medicine, Peiping Union Medical College.  
Received for publication, July 31, 1934.

parison. Recently Greisheimer, Treloar, and Ryan<sup>5</sup> have attempted to show the interrelationship of Cutler, Linzenmeier, and Westergren sedimentation methods, and have provided several tables from which the sedimentation rate or index with any two methods may be read off when the value from one method is known. However, the value of these tables is very limited as they do not provide values for sedimentation times of less than one hour. In other words, these tables do not provide values for predictions of markedly increased sedimentation index. It appears that the standardization of a generally acceptable method for blood sedimentation is highly desirable for hematologic studies, and would certainly prove most advantageous for clinical work.

Rubin and Smith<sup>6</sup> and particularly Rourke and Plass<sup>7</sup> have shown that all other factors being equal, the sedimentation rate bears an inverse proportion to the volume percentage of the red blood cells; the fewer the red blood cells, the faster the sedimentation rate. The influence of variations in cell volume percentage on the sedimentation rate has also been pointed out by Bönniger and Herrmann,<sup>8</sup> Hubbard and Geiger,<sup>9</sup> and Gram.<sup>10</sup> The last mentioned author and Rourke and Ernstene<sup>11</sup> have constructed charts by means of which approximate correction could be made for variations in hemoglobin percentage and cell volume percentage. These charts, however, do not provide any corrections for blood specimens with a hemoglobin percentage less than 70 or over 118, nor for blood specimens with a hematocrit value lower than 20 or over 55. Recently Walton<sup>12, 13</sup> has ingeniously utilized the percentage and dilution formula of Blackloek<sup>14</sup> to standardize the number of the red blood cells of any given blood specimen before performing the sedimentation test. Walton's method undoubtedly constitutes a definite improvement in sedimentation technique. Nevertheless, it is somewhat troublesome, for it requires an enumeration of the erythrocytes in conjunction with each sedimentation test, and in cases with severe anemia it often requires about 10 c.c. of blood to obtain a reliable correction. Furthermore, Walton's method involves the use of sodium citrate solution, which is objectionable because it dilutes the plasma and thus affects the sedimentation rate. As a result of the author's experience with the difficulties in making corrections for the influence of anemia on the sedimentation rate in kala-azar<sup>15</sup> an accurate method, which is much simpler than Walton's, has been developed. This method is based on the fact that the hematocrit is an accurate index of anemia as well as of polycythemia. By utilizing a fixed hematocrit value as standard, the concentration of the red blood cells of any blood can be standardized before performing the sedimentation. That is to say, the correction for the influence of anemia or polycythemia is made before a sedimentation test is performed. Such a direct correction is in itself superior to the indirect corrections obtained by means of reference charts of Gram,<sup>10</sup> and Rourke and Ernstene.<sup>11</sup> For each sedimentation test the actual amount of the blood required is only 1 c.c. whether Linzenmeier's or Westergren's method is followed. However, in order to determine the hematocrit value and to correct the cell volume percentage at the same time before performing the sedimentation test, 3 to 5 c.c. of blood are needed, depending on the severity of the anemia. Usually



about 4 or 5 c.c. of blood are withdrawn from a vein. The blood is then transferred to a hematocrit tube containing 1 to 2 mg. of heparin, which is preferable to inorganic anticoagulants because it does not alter the size of the red blood cells nor the concentration of the plasma proteins. (The hematocrit tubes used by me consist of pipettes sealed flat at one end. These tubes are about 14 cm. in length and have an internal caliber of about 6.5 mm. They are carefully calibrated to 0.01 c.c. and each has a graduated capacity of 5.0 c.c. Other carefully calibrated tubes with about the same capacity may be used.) By repeated inversion of the hematocrit tube about 20 times, the blood is well mixed with the heparin and coagulation prevented. It is frequently necessary to use a small clean glass rod to stir and break up the minute clumps of heparin to secure thorough mixing. A portion of the heparinized blood may now be transferred to a sedimentation tube by a capillary pipette to determine the uncorrected sedimentation rate for comparison with the corrected rate to be ascertained. The remainder of the blood in the hematocrit tube is centrifuged at a speed of 3,000 revolutions per minute for thirty minutes. This is sufficient to pack the cells to a constant volume incapable of further reduction. The volume of the cells is now read and the hematocrit value calculated. The excessive supernatant fluid is then removed, or (as in cases of polycythemia or dehydration) more of the patient's plasma is added with a capillary pipette until the ratio between the volume of the plasma and that of the cells is 1 to 1. The hematocrit value is 50 when this ratio exists. The blood is then thoroughly remixed by inverting the hematocrit tube over and over for ten minutes to ensure even distribution of the cells in the plasma. It is now ready for testing the corrected sedimentation rate regardless of whether the time method, the distance method, or the graphic method is used. That such manipulations of blood do not affect the sedimentation rate is borne out by the work of Rourke, Plass and Ernstene,<sup>7, 11</sup> who showed that blood sedimentation is a completely reversible phenomenon under ordinary laboratory conditions including centrifugation. The author has chosen a hematocrit of 50 as the standard for correction of cell volume, because it is a convenient figure and lies within the upper limit of normal hematocrit values.

TABLE I

FOUR EXAMPLES OF THE APPLICATION OF THE AUTHOR'S METHOD OF CORRECTION TO THE ERYTHROCYTE SEDIMENTATION TEST

CASE	AGE YR.	SEX	DIAGNOSIS	R.B.C. MIL- LIONS	HEMO- GLOBIN		HEM- ATO- CRIT VALUE	UNCORRECTED SEDIMENTA- TION		CORRECTED SEDIMENTA- TION (WRITER'S METHOD)	
					PER CENT	GM. PER 100 G.C.		TIME MIN.	DIS- TANCE MM.	TIME MIN.	DIS- TANCE MM.
1	4	F	Kala-azar	1.7	33.0	4.8	15.7	8	39	65	17
2	34	F	Microcytic hypo- chronic anemia	1.2	13.8	2.0	9.2	10	40	142	11
3	33	M	Aplastic anemia	1.4	24.1	3.5	13.3	10	41	240	10
4	18	M	Banti's disease	2.5	36.5	5.3	18.4	18.5	35	360	6.5

Opinion may differ as to what hematocrit value should be chosen as the normal average standard for correction of anemia or polycythemia. The following formula will be helpful in calculating the volume of the cells and plasma (X) required for a given hematocrit value and a known cell volume when any hematocrit value other than 50 is employed for standardization of the cell volume:

$$\frac{\text{Observed packed cell volume}}{X} = \frac{\text{Hematocrit value desired}}{100} \text{ or } X = \frac{\text{Packed cell volume} \times 100}{\text{Hematocrit value desired}}$$

It is only necessary to calculate the value of X and remove the excessive plasma until the volume of the cells and plasma registers the exact value of X, the actual amount of the plasma being X minus cell volume. This formula holds even in cases of polycythemia, in which all that is necessary is to add plasma up to the value X.

It is realized that in blood with either extreme macrocytosis or microcytosis the number of the red cells in a given hematocrit may vary considerably from normal. Under such condition it is true that the above method does not take into consideration the number of the red cells when the blood is converted to a fixed hematocrit value. One must remember, however, that in any given specimen of blood, there are three variable factors among others that may influence the sedimentation, namely, the number, the size, and the hemoglobin content of the red blood cells. For instance, in two specimens of blood both having a hematocrit value of 20, the number of the erythrocytes and the amount of hemoglobin per unit volume of blood may vary considerably. Similarly the number of the erythrocytes as well as the cell volume percentage of two specimens of blood both having a hemoglobin of 8 grams per 100 c.c. of blood, for example, may be quite different. It is also obvious that in two specimens of blood both having the same erythrocyte counts, say 2 millions of red blood cells per c. mm., the cell volume percentage and the hemoglobin may not be the same. Hence the hematocrit value, the hemoglobin, and the number of the erythrocytes of a specimen of blood cannot be standardized simultaneously for a sedimentation test. In order to control these three factors at the same time one may have to do three sedimentation tests on each specimen of blood, for example, a first test after the blood has been standardized to a hematocrit of 50 volume per cent, a second test after the blood has been standardized to a red cell count of 5 millions per cubic millimeter, and a third test after the blood has been standardized to a hemoglobin of 15 grams per 100 c.c. This, however, is time consuming, and not practicable in clinical work.

I have found that Linzenmeier's tubes are satisfactory for sedimentation tests both by the time method and by the distance method. These tubes are 6.5 cm. in length and 5 mm. in internal diameter with a capacity of a little over 1.0 c.c. They are marked at the 1.0 c.c. level and at a point 18 mm. below it. Their bottoms are concave and they hold a column of blood measuring 52 mm. from the bottom up to the 1.0 c.c. mark. The time required for the sedimentation of 18 mm., i.e., the time required for the appearance of a column of clear plasma measuring 18 mm. from the 1.0 c.c. mark, is recorded

as the sedimentation time in minutes, and the distance of sedimentation or the length of the clear plasma column\* at the end of one hour is noted as sedimentation distance in millimeters. Other suitable tubes may be used.

That the method here given for obtaining a corrected sedimentation rate has a much wider application than the method of Gram<sup>10</sup> and that of Rourke and Ernstene<sup>11</sup> can better be appreciated from a few specific examples. The results of the four illustrative cases summarized in Table I demonstrate the usefulness and the advantage of the method. From this table it is clear that the anemia in each of the four cases influences the sedimentation rate significantly, and that in order to be of value the rate must be corrected. But in all these cases neither the method of Gram nor that of Rourke and Ernstene is applicable because the hemoglobin and the hematocrit values are so low that they are beyond the correction range of the charts prepared by these authors. By the technic here described, however, a corrected rate can be easily obtained for each case. Now suppose we are dealing with a case of polycythemia with a red cell count of 7.5 millions per cubic millimeter, a hemoglobin content of 22 grams per 100 c.c., or 150 per cent, and a hematocrit value of 70. Here again the sedimentation rate must be corrected in order to be of value, but a correction is possible neither with the chart of Gram nor with that of Rourke and Ernstene as these charts do not provide corrections for specimens of blood with a hemoglobin above 118 per cent or with a hematocrit over 55 volume per cent. A correction, however, can be easily obtained by my technic.

#### SUMMARY

A simple and accurate method for standardizing the cell volume percentage of blood for erythrocyte sedimentation test is described.

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\*This has been loosely termed the sedimentation index by some investigators.

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## VALUE OF H AND O AGGLUTINATION TECHNIC IN ROUTINE WIDAL EXAMINATIONS\*

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ROSEMARY BOLE, A.B., M.A., COLUMBUS, OHIO

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IN THE last few years interest has increased in a change in agglutination technic for the diagnosis of enteric fever growing out of Felix's<sup>1</sup> theory of qualitative receptor analysis. This theory is based on observations made some years earlier by Smith and Reagh<sup>2</sup> on flagellar and somatic agglutination of the hog cholera bacillus. Felix claims that antigens of the protens, typhoid and paratyphoid group of organisms are not homogeneous but consist of two separate parts; the stable, somatic or O antigen, identified with the body of the organism, and the labile, flagellar or H antigen, identified with the flagella, each giving rise to a corresponding agglutinin.

Whether one accepts Felix's theory of a multiple antigen or together with Craigie<sup>3</sup> and others, questions the existence of two distinct fractions, is quite aside from the easily demonstrated fact that organisms of the enteric group show two dissimilar types of agglutination. Immune sera produced by inoculation of motile strains of the enteric organisms develop two variously acting agglutinins; one the large-flaking H agglutinin which act upon the flagella to produce a rapid agglutination in fluffy masses, and the small-flaking O agglutinins which act upon the somata to produce agglutination slowly in compact granules, dislodged with difficulty from the bottom of the tube. The nonmotile strains, lacking flagella, produce only O agglutinins.

In respect to the typhoids and paratyphoids, the large-flaking H agglutinins indicate the specific infecting organism. O agglutinins are nonspecific and represent the group factor. Felix's view is that the latter indicate response to infection and show close relationship to the course of the disease, the H agglutinins being negligible in this respect. Triple typhoid vaccination develops H agglutinins in high dilution. These may persist for years with gradual decline in titer. Felix<sup>4</sup> and also Stuart and Krikorian<sup>5</sup> failed

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\*From The State Department of Health Laboratories.  
Received for publication, July 31, 1934.

to demonstrate O agglutinins following vaccination but others report them in low dilution. An attack of enteric fever usually results in the production of both types, O often appearing early in the disease. In a proportion of cases only O agglutinins may be demonstrated. Felix<sup>1</sup> states that it is rare to find only H agglutinins throughout the entire course of infection. Non-enteric febrile diseases following vaccination, or a previous typhoid attack, may give rise to an anamnestic reaction; a nonspecific restimulation of H agglutinins exclusively (Felix<sup>1</sup>).

Arguing from the observed facts Felix<sup>1</sup> made the following claims:

1. The Widal reaction resulting from vaccination and that due to enteric fever can be readily distinguished on the basis of type of agglutination observed.

2. If prophylactic vaccination or a previous enteric infection can be excluded, the diagnosis of typhoid is ensured by the appearance of large-flaking agglutinins.

3. Among inoculated persons showing only H agglutinins for typhoid and the paratyphoids, and in the entire absence of small-flaking agglutinins, the disease can be diagnosed as nontyphoid.

4. Among inoculated persons showing both H and O agglutinins, a diagnosis of enteric infection, type undetermined can be made, this based on the presence of O agglutinins, since in inoculated individuals the type specificity of H agglutinins is no longer applicable.

In England and on the Continent a procedure designed to demonstrate both H and O agglutinins has been used for a number of years. It is only recently in this country that the practical value of a more inclusive technic has been investigated. The work of the New York State<sup>6, 7</sup> and Michigan Departments of Health<sup>8</sup> is notable and the present survey of the Coordination Committee on Standard Methods of the American Public Health Association, also the contributions of Dulauey et al.<sup>9, 10</sup>

Our own interest in the need for a change in the time-honored Widal set-up dates back several years when a marked discrepancy was noted in two types of Widal test as carried out by us. In routine procedure all sera received were examined in hanging drop preparation (dilution 1:40) using a live broth culture of typhoid bacilli. Sera giving positive or atypical results were titrated macroscopically (dilutions 1:20—1:320) against the customary antigen; a phenol or tri-cresol killed typhoid suspension. A fair proportion of sera giving positive results with the live culture were completely negative in macroscopic test. This discrepancy is easily explained on the basis of H and O agglutination. A live motile culture against suitable sera reveals both H and O agglutinins. It is recognized, however, that a phenolized or formolized antigen is not adequate for the demonstration of O agglutinins (Felix and Olitzki<sup>11</sup>). Therefore, given a serum containing only O agglutinins, the result is negative with the preserved suspension, although the same serum is positive against the living organism or an antigen designed to demonstrate O agglutinins.

Following this observation, the value of a more inclusive technic for routine use was investigated. The present paper is an analysis of 250 sera, positive microscopically, which were subsequently titrated against both H and O antigens, and on which it was possible to obtain clinical diagnosis and vaccination history.

*Antigens.*—Many laboratories find it impractical for a variety of reasons to use live organisms for agglutination purposes, therefore it has been common practice to adopt a phenolized or formalized suspension for Widal tests. As has been pointed out, this type of antigen indicates only H agglutinins. The suppression of O may be due, according to Felix and Olitzki,<sup>11</sup> to their impairment by formalin, or as in Craigie's<sup>3</sup> view, the bodies of the organisms may be kept apart mechanically by the formalin hardened flagella, thus preventing somatic agglutination. For both H and O readings a second antigen must be included. This double set-up is preferable to a single antigen of live motile organisms. In our opinion, except for hanging drop technic, a live suspension is unsatisfactory rather than impractical. We have found that a formalized suspension gives a more clear-cut H reading than the live culture, probably due to the hardening effect of formalin on flagella. In addition Felix has pointed out the difficulty of making H and O readings in the same tube.

For the present work H antigens for typhoid, paratyphoid A and paratyphoid B were prepared according to the method of the Medical Research Council, London, England,<sup>12</sup> which calls for veal broth formalized suspensions of selected motile strains. For pure O antigen the alcohol method of Bien and Sontag<sup>13</sup> was followed, using Felix's nonmotile strain, typhoid 901. O antigen may also be made by this method from the motile organism since alcohol destroys the flagella, resulting in a pure somatic product.

*Experimental.*—This laboratory has found it a reasonably safe procedure, when dealing with large numbers of sera routinely, to eliminate negative specimens by preliminary microscopic test, with small risk of missing an occasional positive which might not appear in 1:40 dilution. In proof of this, 100 sera, negative microscopically, were titrated macroscopically using H and O antigens. The results were completely negative. On this basis all sera received for Widal test were examined in hanging drop preparation (dilution 1:40) against a motile typhoid culture, maintained for many years by daily broth transfer. Two hundred fifty sera giving complete or partial agglutination in this way were titrated against three H antigens; typhoid, paratyphoid A and paratyphoid B and typhoid O (dilutions 1:20—1:320). Five-tenths cubic centimeter quantities of sera and antigens were set up using a four-hour incubation period in the water-bath at 52° C.—55° C. followed by 37° C. overnight. H readings were made at the four-hour period; O readings after 37° C. incubation. Agglutination was read to the limit of visibility with the naked eye.

Table I gives the classification of these sera on the basis of clinical diagnosis and vaccination history. They are arranged in four groups: typhoid cases, vaccinated and unvaccinated, nontyphoid or undetermined cases, vac-

inated and unvaccinated. Tables II through V record the type of agglutination in the four groups listed above. Table VI is a summary of the foregoing and shows the percentage distribution of the various types of agglutination for each group. The curves in Chart 1 express the same results.

TABLE I  
CLINICAL DIAGNOSIS AND VACCINATION HISTORY

TYPHOID	VACCINATED	NONVACCINATED
205	10	195
NON-TYPHOID OR UNDE- TERMINED		
45	28	17

TABLE II  
TYPHOID CASES NONVACCINATED

NUMBER OF SPECIMENS	TYPE OF AG- GLUTINATION	TITER				
		20	40	80	160	320
117	H+	0	9	20	18	70
	O+	0	8	25	38	46
47	A-					
	B-					
23	H-					
	O+	2	9	11	10	15
6	A-					
	B-					
1	H+	0	3	4	3	13
	O-					
1	A-					
	B-					
1	H+	1		2		3
	O+		2	2		2
1	A+		1	1		4
	B+		1		1	4
1	H+				1	
	O-					
1	A+			1		
	B+				1	
195 Total	H-					
	O+	1				
	A-					
	B+					1

Table II: The majority (60 per cent) of typhoid patients unvaccinated showed both H and O agglutinins at the time of test. Although the tables are not planned to indicate the degree of agglutination at each titer, in most instances as the higher dilutions were approached, H agglutination was more complete than O. Among the 117 typhoid patients developing both H and O agglutinins, 44 per cent gave higher titer for H than O, 24 per cent showed a higher O titer than H, and in 30 per cent the titer was the same for both. In a study of 32 typhoid sera Dulaney<sup>10</sup> found 53 per cent giving higher O titer than H; in 12 per cent H exceeded O and 31 per cent gave approximately the same titer for H and O. One serum developed neither agglutinin. The predominance of higher O titers in Dulaney's series is probably due to the

fact that she used a longer incubation period at 52° C., twenty-four hours. In our series a proportion of sera gave only H (11.7 per cent) or only O (24 per cent) agglutinins. It is recognized that O agglutinins develop early in the course of infection. Later tests doubtless would have revealed the specific H antibody in some instances. However, if a technic using only a formalized or phenolized H antigen had been followed, the laboratory result of necessity would have been negative. With but one opportunity to present a report, the error would have been considerable. A small percentage (3.5) of sera developed H agglutinins for all three types. Misinformation as to vaccination history is a possible explanation here, although Felix<sup>4</sup> notes an occasional nonvaccinated case showing multiple H agglutinins. The one serum giving

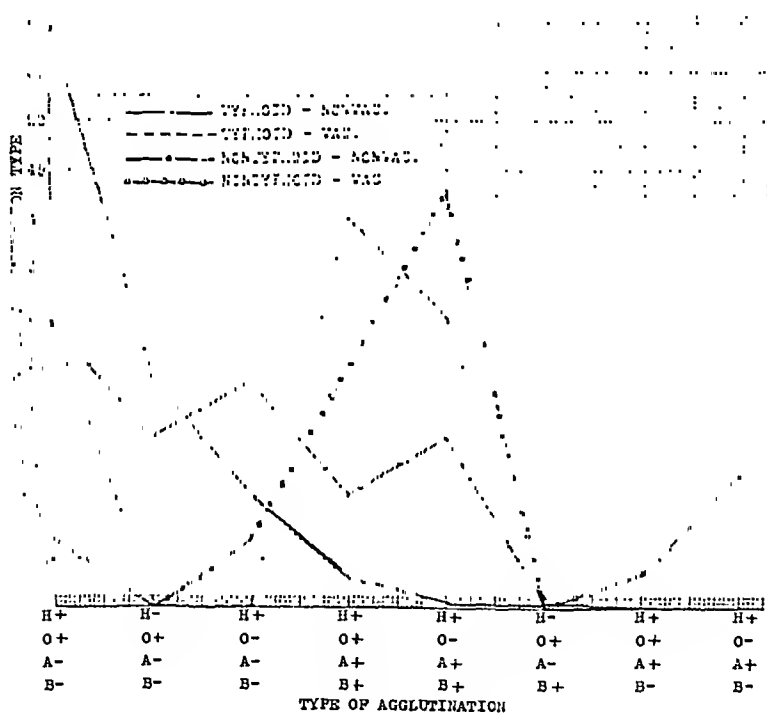


Chart 1.—Agglutination types in four classes of sera.

O agglutination and paratyphoid B in the specific H type suggests paratyphoid infection although the organism was not isolated.

*Table III:* The number of patients in the typhoid vaccinated group is small. As is to be expected, a proportion (40 per cent) gave all four types of agglutination. Since vaccination complicates the serologic picture, the laboratory diagnosis would be limited to a statement of enteric infection of undetermined type, this on the basis of O agglutination.

*Tables IV and IVA:* In the nontyphoid nonvaccinated group many combinations of the agglutination picture are found. In most instances the patient's history helps to give an explanation of the results. Table IVA is a detailed record of the 17 sera included in this group. Natural agglutinins



TABLE III  
TYPHOID CASES, VACCINATED

NUMBER OF SPECIMENS	TYPE OF AG-GLUTINATION	TITER				
		20	40	80	160	320
3	H+	1	1	1	1	1
	O+					1
	A-					
	B-					
4	H+			1		4
	O+					3
	A+					3
	B+					2
3	H+		1			2
	O-					
	A+					2
	B+					1
10 Total						

may explain those of very low titer. In addition there are doubtless typhoid cases unrecognized clinically. Nos. 1 and 3 well may be of this class. Previous typhoid history accounts for the results in at least two (Nos. 5 and 13). Of interest are the four sera giving agglutination results with *Brucella abortus* (Nos. 7, 10, 11, 12) three of which are definitely diagnosed as undulant fever, and serum No. 16 in which the Wassermann and Kahn are positive. There are repeated records in the literature to indicate that many nonrelated infections may stimulate the production of typhoid agglutinins. Gilbert and Coleman<sup>14</sup> record the results on 150 undulant fever cases. A proportion of these with no history of vaccination or enteric infection gave definite typhoid

TABLE IV  
NONTYPHOID OR UNDETERMINED CASES NONVACCINATED

NUMBER OF SPECIMENS	TYPE OF AG-GLUTINATION	TITER				
		20	40	80	160	320
5	H+	1	1	2	1	3
	O+					1
	A-					
	B-					
3	H-	1	1		1	
	O+					
	A-					
	B-					
4	H+		1		2	1
	O-					
	A-					
	B-					
2	H+	1		1	1	1
	O+					
	A+					
	B+					
3	H+		1	1	2	1
	O-					
	A+					
	B+					
17 Total						

agglutination. The possibility of inaccurate vaccination history is a consideration in at least three cases (Cases 14, 15, 17). Case 14 is a nurse who has doubtless been vaccinated. Cases 15 and 17 are men of military age who may have had war service and therefore triple typhoid vaccination.

TABLE IVA  
NONTYPHOID OR UNDETERMINED CASES NONVACCINATED

NUMBER	NAME	TYPE OF AGGLUTINATION				CLINICAL DIAGNOSIS	REMARKS
		TYPHOID H	TYPHOID O	PARA A	PARA B		
1	R. S.	+ 1:320	+++ 1:160	-	-	Mixed infection	Exposure to malaria
2	M. D.	++ 1: 20	++ 1: 80	-	-	Influenza	
3	L. J.	++++ 1:320	++++ 1:320	-	-	Ethmoid cell infection	Temp. 103, white count 4,300
4	Mrs. B.	+++ 1:160	++ 1: 40	-	-	Influenza	
5	J. C.	++++ 1:320	+ 1: 80	-	-	Typhoid carrier	Typhoid 2 years ago
6	E. B.	-	++ 1: 40	-	-	Persistent low fever	
7	D. A.	-	+++ 1:160	-	-	Cystitis	Agg. with Br. Abortus 1:40
8	A. H.	-	++ 1: 20	-	-	Influenza	
9	J. H.	+ 1:160	-	-	-	Perineal abscess	
10	A. J.	+ 1:160	-	-	-	Undulant fever	Agg. with Br. Abortus +1:2560
11	C. G.	++++ 1:320	-	-	-	Undulant fever	Agg. with Br. Abortus +1:40
12	R. H.	++++ 1: 40	-	-	-	Undulant fever	Agg. with Br. Abortus +1:2560
13	Mrs. T.	+++ 1: 80	+ 1: 20	++ 1:160	+ 1: 40	Questionable	History of typhoid in childhood
14	D. S.	++ 1:320	+ 1: 80	++ 1: 80	++++ 1: 40	Questionable	A nurse
15	L. R.	++++ 1:160	-	+++ 1: 80	++++ 1: 40	Empyema	Male Age 49
16	L. B.	++++ 1:320	-	++ 1:320	+ 1:320	Pyelitis	Wassermann ++ Kahn ++++
17	M. E.	+++ 1:160	-	+++ 1: 40	++++ 1:320	Undetermined	Male Age 40

Table V: H agglutinins predominate in the nontyphoid vaccinated groups; 64.2 per cent showed the H type only, usually for typhoid, paratyphoid A and paratyphoid B, since triple typhoid vaccination is common; 35.7 per cent developed O agglutinins also but in no case did the O titer exceed 1:80. Both Gardner<sup>15</sup> and Dulaney<sup>9</sup> report a higher titer than this in vaccinated patients. However, all workers are agreed that the O titer following vaccination does not reach the levels developed by an attack of enteric fever.

*Relation of Agglutination Results to Positive Blood Culture.*—Of the 250 specimens under consideration, blood cultures were made on 118. After centrifuging, the clots were cultured overnight in peptone bile and streaked on Endo plates the following day. Typhoid bacilli were isolated in 29 or 24.9 per cent. Although these specimens are submitted primarily for serologic test and often do not reach the laboratory in ideal condition for culture, there is very evident value in including a blood culture as part of the diagnostic technic on specimens intended for Widal test. Table VII lists the positive

cultures and shows their relation to agglutination results. Fourteen (48 per cent) gave both H and O agglutination, two (7 per cent) H only, eleven (37 per cent) O only, and two (7 per cent) agglutinated in all four types. The results show that it is possible to obtain a positive blood culture after both H and O agglutinins have developed. Of particular interest is the large proportion of positive cultures where the sera showed O agglutinins only. These are doubtless early cases. It is known that O agglutinins appear early in the disease and positive cultural findings are also associated with this

TABLE V  
NONTYPHOID CASES VACCINATED

NUMBER OF SPECIMENS	TYPE OF AG-GLUTINATION	TITER				
		20	40	80	160	320
2	H+ O+ A- B-		2			2
2	H+ O- A- B-				2	
7	H+ O+ A+ B+	1 1 1	1 5 2 2	1 1 1 3	1 1	3 2 2
12	H+ O- A+ B+		2 3 2		4 3 2	6 5 4
1	H+ O+ A+ B-		1			1 1
4	H+ O- A+ B-		2	2		2 2
28 Total						

TABLE VI  
AGGLUTINATION TYPES IN FOUR CLASSES OF SERA

HISTORY	TYPE OF AGGLUTINATION								TOTAL
	H+ O+ A- B-	H- O+ A- B-	H+ O- A- B-	H+ O+ A+ B+	H+ O- A+ B+	H- O+ A- B+	H+ O+ A+ B-	H+ O- A+ B-	
Typhoid Nonvaccinated	117 60.0%	47 24.1%	23 11.7%	6 3.0%	1 0.5%	1 0.5%			195
Typhoid Vaccinated	3 30.0%			4 40.0%	3 30.0%				10
Nontyphoid Nonvaccinated	5 29.4%	3 17.6%	4 23.5%	2 11.7%	3 17.6%				17
Nontyphoid Vaccinated	2 7.1%		2 7.1%	7 25.0%	12 42.8%		1 3.5%	4 14.2%	28
				*					250

TABLE VII  
AGGLUTINATION TYPES ASSOCIATED WITH POSITIVE BLOOD CULTURE (*B. Typhosus*)

NUMBER	TYPE OF AGGLUTINATION AND TITER				REMARKS
	TYPHOID H	TYPHOID O	PARA A	PARA B	
1	1-320	1-160	-	-	
2	1- 80	1- 40	-	-	
3	1- 80	1- 80	-	-	
4	1- 20	1-160	-	-	
5	1-160	1- 40	-	-	
6	1-320	1-320	-	-	
7	1-320	1-320	-	-	
8	1- 40	1- 80	-	-	
9	1- 80	1- 40	-	-	
10	1-160	1- 40	-	-	
11	1-320	1-320	-	-	
12	1-160	1-160	-	-	
13	1- 40	1-160	-	-	
14	1- 80	1- 80	-	-	
15	1-320	-	-	-	
16	1- 80	-	-	-	
17	-	1- 40	-	-	
18	-	1- 20	-	-	
19	-	1- 20	-	-	
20	-	1-320	-	-	Third week of illness
21	-	1-320	-	-	
22	-	1- 40	-	-	
23	-	1- 80	-	-	
24	-	1- 40	-	-	
25	-	1- 80	-	-	
26	-	1-160	-	-	Seventh day of illness
27	-	1- 40	-	-	
28	1-320	1-320	1-320	1-320	Fourteenth day of illness Vaccinated 8 wk. previously
29	1- 80	1- 40	1- 40	1- 40	No vaccine

period. Aside from indicating the importance of including a blood culture with the serologic test, these results emphasize the value of O technic in routine procedure and its diagnostic importance even in very low titer, particularly so when vaccination can be excluded. Eight of the eleven specimens giving positive blood culture and showing O agglutinins only, developed a titer of 1:80 or under. In two the titer limit was 1:20.

#### DISCUSSION

In a laboratory receiving Widal specimens from a wide geographic area, it usually happens that but one blood sample is received throughout the course of illness. Information concerning clinical diagnosis, time of onset and vaccination history invariably is meager if given at all. Follow-up information or additional specimens are difficult to obtain. This gives no opportunity to apply Dreyer's technic to trace the fluctuation of agglutinins, a method claimed to have diagnostic value. Under these conditions it is essential that some method of laboratory diagnosis be adopted which will give the greatest amount of information both to the doctor and the laboratory. We feel that this can be accomplished by eliminating negative sera by microscopic test and titrating all other specimens against H antigens for typhoid, paratyphoid A and paratyphoid B and typhoid O. \*In addition, the clot should be cultured.

In this way it is possible, within limits, to differentiate vaccination cases from those of enteric fever and to make serologic diagnosis at any stage of the disease at which agglutinins of whatever type are developed.

For diagnostic purposes Felix suggests a single dilution of 1:100 or 1:200 against H and O antigens as adequate procedure in the majority of instances. In discussing O agglutinins Gardner sets an O titer of about 1:200 as probably diagnostic of infection regardless of vaccination. Dulaney suggests 1:500 to 1:1000 as strong indication of specific disease. These figures doubtless vary somewhat with the sensitiveness of the antigen and other conditions of the test. In our opinion serial titration with all four antigens is essential when but one test can be made. A range from 1:20 through 1:320 is sufficiently wide for most sera. It is probably sufficiently high to exclude nonspecific O agglutinins and the low titers will indicate very early cases which might escape detection with but one mean dilution. If all three H antigens are used, it is unnecessary to extend the H titration to the end-point, since multiple H agglutination in reasonable titer usually is an indication of vaccination.

#### SUMMARY

H and O agglutination results are presented on 250 sera submitted for Widal examination, and the results of blood culture on the same specimens are given in a proportion of these. By the use of multiple antigens it is possible, within limits, to distinguish vaccination cases from those of enteric infection. The majority of nonvaccinated typhoid cases develop both H and O agglutinins. Either type may be present alone. Triple typhoid vaccination stimulates the production of H agglutinins in high titer; O agglutinins may be present but in comparatively low dilution.

Based on these results a routine Widal procedure is suggested for laboratories handling large numbers of sera and having but one opportunity of testing a given specimen. By this method negative sera are eliminated in microscopic test with a live typhoid culture. The remainder are titrated against formolized H antigens (typhoid, paratyphoid A, and paratyphoid B) and alcoholized typhoid O. A dilution series from 1:20 through 1:320 usually is adequate to rule out nonspecific O agglutinins and to indicate very early cases. Blood culture is recommended on the same specimen used for serologic test.

Acknowledgment is given to Calvin Kersell, Assistant Serologist, for valuable service in preparing antigens, and setting up many hundreds of tests from which those under discussion were selected.

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## DETERMINATION AND RECOGNITION OF LEAD IN BIOLOGIC TISSUES AND FLUIDS\*

C. N. MYERS, PH.D., SC.D., FLORENCE GUSTAFSON, M.A., AND  
BINFORD THRONE, M.D., NEW YORK, N. Y.

**D**URING the past decade the clinician has been confronted with many problems in the diagnosis of obscure symptoms and complexes. The problem of accurate diagnoses has been made more difficult on account of these complex factors associated with modern changes in our daily existence and environment. It is scarcely necessary to call attention to the daily visits of many thousands of patients who return from their professional visit with uncertain minds as to the causative factors concerned with their disturbed metabolism. Many neurologic symptoms associated with brain physiology and the associated involuntary nervous system, many problems of internal character associated with the "so-called" reticulo-endothelial system, as well as dermatologic lesions hitherto unexplained, have recently been associated with the intake of metals and their resultant pathology. Such clinical syndromes as dermatitis, endarteritis, Raynaud's disease, various muscular dystrophies and atrophies, and particularly reticulo-endothelial dysfunctions, have been directly associated with this metallic retention.

Time and space will permit only a brief reference to some of our previous studies. It has been emphasized by us that in our present mode of living ingestion and contact absorption of heavy metals constitutes a serious public health hazard. It is our belief that these studies first called attention to a

\*Received for publication, July 20, 1934.

third type of toxemia which has been designated as the "subchronic stage" which is associated with the cumulative action of small amounts of metal. This syndrome may require many weeks or even years before the serious character of this cumulative action manifests itself. The small amounts of metal accumulate in areas of cell damage. It may be a psoriatic lesion, a traumatized area, a diseased liver, locations of disease or any other injured part, and, in these, the metals readily accumulate serving as the fulminating factor. Our concern has been chiefly with arsenic, lead, nickel, and mercury.

The common sources of exposure will be described in detail in other articles dealing with the clinical results. The present study will deal with the detection of lead in organic material, and a discussion of interfering substances. Our clinical problem concerns not the plumbism associated with massive doses or contacts yielding acute symptoms, but, on the contrary, it is associated with the little known, a slow, subtle, insidious saturation of the system by infinitesimal doses extending over a long period of time and producing a group of symptoms altogether different from the recognized forms of plumbism. Industrial lead poisoning ranks second in New York state, and the place of our "subchronic stage" has yet to be allotted recognition notwithstanding its wide prevalence. The results being presented open the way to a discussion of the "so-called" normal lead content, and then the relation of this retained lead will be discussed in connection with arthralgia and neuritis, muscular atrophy, cancer, and dysfunction of the vegetative nervous system.

Many clinicians have found that the manifestations of lead poisoning may resemble those which result from other organic diseases and that a differential diagnosis on the basis of their clinical findings alone has been difficult. In the study of some of these obscure cases the laboratory has been able to furnish the medical profession with additional information. From the survey of the literature it has been found that the methods for the quantitative detection of minute quantities of lead in biologic fluids and tissues are few, although criticisms and modifications are found in abundance. However, a careful review will show that there is usually some modification of the Fairhall method. It is our purpose here to restrict ourselves purely to the analytical study of this lead procedure and its application to biologic material. The method as reported in this research will furnish the basis of the data which will be used in our future clinical reports.

Our studies do not include the isolation of minute quantities of lead by means of electrochemical, microscopic, or spectrographic methods. The procedures which have been followed have been based upon Fairhall's volumetric and colorimetric estimations.

#### DETERMINATION OF LEAD IN BIOLOGIC MATERIAL

##### I. Preparation of Samples.—

A. *Urine*: A liter of urine is used whenever possible. The urine must be fresh or preserved, preferably, with thymol. It is made ammoniacal by the addition of 50 c.c. of concentrated ammonium hydroxide and allowed to stand overnight. The lead is carried down by entrainment in the phosphate precipitate. Most of the supernatant fluid may be siphoned

off and the rest of the sample is filtered through 12.5 cm. No. 40 Whatman filter paper. The specimen is dried in the oven and is then ready for ashing and extraction.

B. *Blood*: Whenever possible, 10 c.c. of oxalated blood are measured into a weighed Coors porcelain crucible and dried in the oven. Clot may be used. When the specimen is dry it is weighed before ashing to determine dry weight.

C. *Feces*: The sample is placed in a weighed Coors porcelain crucible, dried, and weighed before ashing.

D. *Spinal Fluid*: A measured volume is placed in a crucible, weighed and dried in the oven before ashing.

E. *Hair and Nails*: Weigh and ash.

F. *Tissue*: Dry, weigh and ash.

## II. *Ashing and Extraction*.—

The samples are ashed as completely as possible at red heat over a Bunsen flame. About 1 c.c. of fuming nitric acid may be added to the urine, feces, and blood samples to complete the ashing. This is not necessary with spinal fluid, hair, nails, and tissue.

The crucibles are cooled and 7 c.c. of 1:2 hydrochloric acid are added. The acid is boiled gently in the crucibles, cooled and filtered through 7 cm. No. 40 Whatman filter paper into a 250 c.c. Pyrex Erlenmeyer flask. The crucibles are washed four or five times with approximately 5 c.c. of cold, distilled water for each washing. The washings are added to the filtrate and the final volume is about 35 c.c.

The filtrate is made just alkaline with approximately 5 c.c. of 25 per cent sodium hydroxide, then just acid with 1:2 hydrochloric acid and 1 c.c. of acid is added in excess. Methyl orange is used as an indicator. The solution is diluted to about 150 c.c. and hydrogen sulphide is passed into the cold solution for thirty minutes. The sample is then allowed to stand overnight to insure complete precipitation of lead sulphide.

The sulphide solution is filtered through 12.5 cm. No. 40 Whatman filter paper. The flasks and filters are washed three times. About 15 c.c. of distilled water are used for each washing. Fifteen cubic centimeters of hot 1:1 nitric acid are used to dissolve the lead sulphide precipitate and this is caught in the precipitation flasks. The filters are washed with hot water until the total volume in the flasks is about 50 c.c. This solution is evaporated down to about 5 c.c. and transferred quantitatively to a 150 c.c. Pyrex beaker. The total volume of solution and washings should be about 50 c.c. Three drops of phenolphthalein are added and the sample made just alkaline with 25 per cent sodium hydroxide, just acid with 10 per cent acetic acid and 1 c.c. of acid is added in excess. The contents of the beaker are boiled, then 1 c.c. of 1 per cent potassium chromate is added and then heated on the water-bath for one hour, then allowed to stand overnight.

The chromate is filtered hot through 7 cm. No. 40 Whatman filter paper, then both beaker and filter are washed three times, each with hot water using about 10 c.c. for each washing. The chromate precipitate is dissolved off the filter paper with 2 c.c. 1:2 hydrochloric acid which is caught in the precipitation beaker. The filter is washed with 40 c.c. of water added in small portions. Five cubic centimeters of 10 per cent potassium iodide are added to each sample and after three minutes they are titrated with 0.005 N sodium thiosulphate, using starch as an indicator. One cubic centimeter of 0.005 N sodium thiosulphate equals 0.3451 mg. lead. A microburette used for the titration is graduated in 1/100 c.c. graduation. The 10 per cent potassium iodide solution is made up fresh each time. The sodium thiosulphate solution is made up fresh each time from a stock solution of 0.1 N thiosulphate. The keeping and standardization of the stock solution of sodium thiosulphate may be found in any good quantitative analysis book.

The method outlined can be used for the determination of lead in various kinds of biologic material. The accuracy of this method of analysis was tested



by the addition of known amounts of lead to the different biologic fluids and tissues. The amount of lead recovered and the error of determination will be found in Tables I, II, and III.

In analyzing the results of the tables it will be noted that the error is more or less constant. The error is not proportional to the quantity of lead determined, and it is felt that it is largely an error of end point. In a review of the literature it was found that Fairhall, in his analyses, observed this same condition. In our earlier work on lead it was found that by using a burette graduated to 0.1 c.c. divisions, a much larger error due to titration was introduced. In our present work a microburette graduated to 0.01 c.c. divisions is used.

In Table II will be found the results obtained by several of our laboratory workers. Even these results do not warrant the criticism that is found in the literature. The greatest errors in this particular study were found when 0.5 mg. of lead was added to the biologic specimen to be analyzed. In passing, it should be said that this method may be used by various workers in the

TABLE I  
RECOVERY OF LEAD IN BIOLOGIC MATERIALS  
VOLUMETRIC METHOD

MATERIAL	AMOUNT C.C.	LEAD ADDED MG.	LEAD FOUND MG.	ERROR
Urine*	800	0.05	0.046	-0.004
	800	0.05	0.065	+0.015
	800	0.05	0.058	+0.008
	800	0.10	0.103	+0.003
	550	0.10	0.095	-0.005
	800	0.10	0.108	+0.008
	800	0.10	0.085	-0.015
	500	0.15	0.172	-0.022
	800	0.15	0.145	-0.005
	800	0.20	0.195	-0.005
	800	0.20	0.213	+0.013
	800	0.20	0.208	+0.008
	800	0.20	0.182	-0.018
	800	0.20	0.196	-0.004
	800	0.20	0.191	-0.009
	800	0.20	0.205	+0.005
	800	0.20	0.2001	+0.0001
	800	0.20	0.191	-0.009
	800	0.25	0.235	-0.015
	800	0.30	0.336	+0.036
AVERAGE ERROR $\pm 0.001$				
Blood	10	0	0	0
	10	0.05	0.48	-0.002
	10	0.05	0.495	-0.005
	10	0.10	0.116	+0.016
	10	0.10	0.083	-0.017
	10	0.15	0.143	-0.007
AVERAGE ERROR $\pm 0.003$				
Spinal fluid	5	0.05	0.041	-0.009
	5	0.05	0.041	-0.009
	5	0.05	0.043	-0.007
AVERAGE ERROR - 0.003				

\*The amount of lead found represents the total lead recovered minus the lead present in the composite sample of urine.

The buret used in these determinations was graduated in 0.01 c.c. divisions.

laboratory and the results are very much in accordance with each other. This fact alone is of great importance because so many methods, which are reported, seem to be carried out successfully only by the person or persons who originally worked on the method.

TABLE II  
RECOVERY OF LEAD IN URINE\*  
VOLUMETRIC METHOD

LEAD ADDED MG.	LEAD FOUND MG.	ERROR	LEAD ADDED MG.	LEAD FOUND MG.	ERROR
0.10	0.11	+0.01	0.25	0.21	-0.04
0.10	0.10	0	0.25	0.20	-0.05
0.10	0.09	-0.01	0.25	0.19	-0.06
0.10	0.09	-0.01	0.25	0.19	-0.06
0.10	0.07	-0.03	0.25	0.19	-0.06
0.10	0.11	+0.01	0.25	0.19	-0.06
0.10	0.12	+0.02	0.25	0.27	+0.02
0.125	0.08	-0.045	0.25	0.27	+0.02
0.125	0.08	-0.035	0.25	0.23	-0.02
0.25	0.22	-0.03	0.25	0.21	-0.04
0.25	0.21	-0.04	0.25	0.20	-0.05
0.25	0.19	-0.06	0.50	0.39	-0.11
0.25	0.26	+0.01	0.50	0.36	-0.14
0.25	0.24	-0.01	0.50	0.37	-0.13
0.25	0.28	+0.03	0.50	0.39	-0.11
0.25	0.22	-0.03	0.50	0.35	-0.15
0.25	0.19	-0.06	0.50	0.40	-0.10
0.25	0.22	-0.03	0.50	0.42	-0.08
0.25	0.26	+0.01	0.50	0.38	-0.12
0.25	0.24	-0.01	0.50	0.35	-0.15
0.25	0.20	-0.05			

\*500 c.c. of urine used in each determination. Buret used in these titrations was graduated in 0.1 c.c. divisions.

In our study of the presence of minute quantities of lead in biologic material, the possible presence of interfering metals was realized and their effect investigated. At the present time these complicating substances have been added to both fresh and old urine. It was found in this research that copper and bismuth are the two metals which cause the greatest interference.

Table III gives not only the amounts of lead detected in the presence of other substances, but, also, additional results showing the amount of lead which has been recovered from urine to which known amounts of lead had been added. These recoveries also show that the technic of the method is improved with constant manipulation, and that these results prove the accuracy of the Fairhall method when adapted to minute quantities of lead in biologic material.

So many reports in the literature state that the volumetric method, as devised by Fairhall, could not detect the minute quantities which would be found in biologic material, and suggestions were given for the use of colorimetric determinations. It has never been our opinion that the results from colorimetric procedures could compare with volumetric, but, nevertheless, to convince ourselves in regard to this point in connection with our analytical studies of lead these determinations were carried out. The method used is essentially the same as described in the first part of this paper up to the point

TABLE III  
LEAD IN URINE IN THE PRESENCE OF OTHER METALS  
VOLUMETRIC METHOD

LEAD ADDED MG.	OTHER METALS ADDED MG.	LEAD FOUND MG.	ERROR
0.20		0.2001	+0.0001
0.20		0.2001	+0.0001
0.20	0.05 mg. Cu	0.188	-0.012
0.20	0.10 mg. Cu	0.203	+0.003
0.20	0.15 mg. Cu	0.214	+0.014
0.20	0.20 mg. Cu	0.215	+0.015
0.20	0.25 mg. Cu	0.196	-0.004
0.20	0.30 mg. Cu	0.133	-0.067
0.20	0.50 mg. Cu	0.176	-0.024
0.20	1.00 mg. Cu	0.207	+0.007
0.20	10.00 mg. Cu	0.257	+0.057
0.20	20.00 mg. Cu		
0.20	30.00 mg. Cu	Cu interfered	
0.05		0.065	+0.015
0.10		0.108	+0.008
0.20		0.191	-0.009
0.20		0.191	-0.009
0.20	0.20 mg. Fe	0.148	-0.052
0.20	0.25 mg. Fe	0.2002	+0.0002
0.20	0.30 mg. Fe	0.207	+0.007
0.20	0.50 mg. Fe	0.193	-0.007
0.20	1.00 mg. Fe	0.2002	+0.0002
0.20	10.00 mg. Fe	0.162	-0.038
0.20	20.00 mg. Fe	0.2001	+0.0001
0.20	30.00 mg. Fe	0.2001	+0.0001
0.20	50.00 mg. Fe	0.210	+0.010
0.20		0.191	-0.009
0.20	20.00 mg. Zn	0.191	-0.009
0.20	20.00 mg. Hg	0.205	+0.005
0.20	20.00 mg. Ca	0.191	-0.009
0.20	20.00 mg. Bi	Bi interfered	
0.20		0.191	-0.009
0.20	0.05 mg. Fe	0.210	+0.01
	0.05 mg. Cu		
0.20	0.10 mg. Fe	0.188	-0.012
	0.10 mg. Cu		
0.20	0.15 mg. Fe	0.217	+0.017
	0.15 mg. Cu		
0.20	0.20 mg. Fe	0.210	+0.01
	0.20 mg. Cu		
0.20*		0.155	-0.045
0.20*	30.00 mg. Ca	0.145	-0.055
0.20*	40.00 mg. Ca	0.148	-0.052
0.20*	30.00 mg. Zn	0.184	-0.016
0.20*	5.00 mg. Bi	0.114	-0.086
0.20*	10.00 mg. Bi	Bi interfered	
0.20*	100.00 mg. Fe	0.213	+0.013
0.20*	30.00 mg. Hg	0.174	-0.026

\*Urine specimens were old and no preservative had been added.

where all the soluble chromate is removed by filtration and repeated washings. The procedure from there on is described in the following paragraphs:

#### COLORIMETRIC PROCEDURE

This residue is then dissolved in 7 c.c. of cold 1:2 HCl and the solution caught in the precipitation beaker. The acid is allowed to rinse the sides of the beaker and is then transferred to a 50 c.c. volumetric flask. The paper and beaker are rinsed with several portions of cold water which are also added to the flask until it contains about 40 c.c.; 1 c.c. of diphenyl carbazide is added and the volume made up to 50 c.c. The best results are obtained when the color is that given by about 0.1 mg. of lead. The standard is prepared in another

50 c.c. flask by adding 7 c.c. of 1:2 HCl, water up to 40 c.c., 1 c.c. of standard lead chromate solution (1 c.c. = 0.1 mg. lead), 1 c.c. of diphenyl carbazide and water up to 50 c.c. The unknown and standard are compared in a colorimeter.

It is important that the standard and the unknown be nearly the same color. This can be arranged by varying the amount of standard lead chromate used or by diluting the unknown. If the unknown proves to have much less than 0.1 mg. of lead, another standard should be made adding all reagents except the lead chromate and adding that drop by drop until the resulting color is similar to that of the unknown. Note the total amount of lead chromate solution added.

If the unknown appears to contain a much larger quantity of lead than 0.1 mg. (as shown by the amounts of sulphide and chromate precipitate), the acid solution of the chromate may be made up to 50 c.c. with water and an aliquot of that solution diluted again, so that the amount of lead in a 50 c.c. volume will be from 0.1 to 0.2 mg. When this is done, more acid should be added to the final dilution to make the total amount equivalent to that in the standard. The setting of the standard in the colorimeter varies with the depth of color. The standard can be matched against itself at different positions to find which is most suitable. The lighter shades are more easily matched.

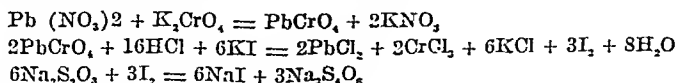
The reagent used to develop the color is a 1 per cent solution of S-diphenyl carbazide in glacial acetic acid. The standard lead chromate solution was prepared by dissolving 0.1560 gm. pure lead chromate in 200 c.c. of 10 per cent HCl and diluting to a liter with water. 1 c.c. = 0.1 mg. lead. This solution may be checked against a standard potassium dichromate solution. The use of the reagent S-diphenyl carbazide may offer a satisfactory solution determination of lead, but, on account of the impure form in which most of it is obtained, the accuracy is open for discussion. The purification of this reagent is a very important factor so far as colorimetric lead methods are concerned.

#### *Calculation.—*

Reading of Standard/Reading of Unknown\* × Mg. Lead in Standard\* = Mg. Lead in Sample.

#### FAIRHALL METHOD—(ABSTRACT OF ORIGINAL VOLUMETRIC METHOD)

1. Ashed at below red heat.
2. Excessive and prolonged heating avoided and ashing accelerated by extracting residue with HCl and hot H<sub>2</sub>O. Uses tartaric acid plus few drops of HCl to dissolve remaining residue. (Tartaric acid must be tested for lead.)
3. Neutralize with NaOH using methyl orange as an indicator and finally adding just sufficient HCl to give a distinct acid reaction. Avoid excess acid since incomplete precipitation of lead may occur in the following step.
4. Saturate lead with hydrogen sulphide to precipitate the lead sulphide. After filtering, precipitate should be washed with boiled distilled H<sub>2</sub>O and dissolved in from 2 to 5 c.c. of concentrated nitric acid. The solution of lead nitrate is then boiled to expel the hydrogen sulphide. It is then neutralized with NaOH and acidified with acetic acid. Excess of potassium chromate is added and solution boiled and allowed to stand overnight. The precipitated lead chromate should be thoroughly washed with warm water and finally should be washed as completely as possible from the filter paper into the flask. The filter paper should be washed with 2 to 5 c.c. of 1:1 HCl followed with warm water to remove last traces of chromic acid. The lead chromate readily dissolves in HCl and the lead may then be estimated by adding an excess of potassium iodide and titrating the iodine liberated by the action of the chromic acid with 0.005 N sodium thiosulphate.



\*Diluted to 50 c.c.

## COLORIMETRIC

The lead chromate was dissolved in HCl, 1 c.c. of a solution of S-diphenyl carbazide in glacial acetic acid and the whole solution diluted to a given volume and determined colorimetrically.

Recently, the Department of Agriculture has made a rather extensive study of the determination of lead in foods. Their methods have been described and it is of particular interest to refer to this development because of the fact that there are six methods for the determination of small quantities of lead described. These methods are presented in the order of their development and their practicability increases with the development. The limiting factors of the electrolytic separation and the iodometric determination of lead are the magnitude of the anode or oxygen "blanks" and the accuracy of the iodometric titrations.

## DISCUSSION

The small amount of lead present in biologic materials requires careful technic and special precautions so that no contamination will occur. The amounts of lead which may be introduced in reagents and apparatus, although small, become of importance when compared to the amount of lead actually present in biologic material. In all of the work blank determinations on reagents and apparatus have been made. In our earlier work a quartz crucible was used but because of the great number of determinations which were carried out this was abandoned in favor of the Coors porcelain crucible which was found to contain no lead. A blank is carried out with each set of determinations to eliminate any possible contamination from this source. In our research the only reagent in which traces of lead were sometimes found was the sodium hydroxide. It was found that the Whatman filter paper was very satisfactory and contained no lead when carried through the procedure as outlined in the first part of this paper. The usual precautions are taken when ashing in regard to excessive heating. Complete drying of specimen before ashing is essential. The adjustment of acidity by means of methyl orange is carefully carried out because of the danger of a loss of lead in this step of the method. The hydrogen sulphide is obtained from the Ohio Chemical Company. The usual precautions in regard to the preservation of the sodium thio-sulphate solution are followed. Lead, like other metals, finds certain areas of maximum deposition. Likewise, it is a well established physiological fact that the patient who retains metals is the one who shows the most clinical symptoms. The optimum condition in every patient is that in which the output is equal to the intake. It is the metal which circulates through the blood and bathes the various tissues which causes the greatest injury—whether it is expressed in terms of metabolic dysfunction or neurological manifestations. Consequently, examination of blood, urine, and spinal fluid is the most important mode of attack. The examination of the skin alone for lead is a very poor excuse for a diagnostic procedure. Quantitative distribution of phosphates indicates that three-quarters of the total amount in the body is in the bones. The muscles contain more than the combined total of the central nervous system, skin, liver, and kidneys, thus producing sufficient evidence

of the inability to place very much diagnostic value on skin tests for lead. Lead, for the most part, replaces the calcium in the phosphates. The accuracy and value of the spectrometric method is yet to be established even though the results from day to day are uniform. Such is not the relation in the human economy.

Some procedures of analysis use the method of wet ashing. It is our opinion that this is not as satisfactory as the procedure of slow and careful dry ashing at a low temperature. It, however, is an important item in connection with the interfering substances which may occasionally play a part in the determination of lead. The addition of any type of reagent to the specimen always raises a question as to the purity of the reagent in respect to lead as well as the interfering substances. The character of the interference also plays a part in the determination of lead. This is illustrated in Table III. It will be

TABLE IV  
RECOVERY OF LEAD IN URINE BY COLORIMETRIC METHOD

LEAD ADDED MG.	LEAD FOUND MG.	ERROR	LEAD ADDED MG.	LEAD FOUND MG.	ERROR
0.05	0.04	-0.01	0.25	0.19	-0.06
0.10	0.07	-0.03	0.25	0.17	-0.08
0.10	0.06	-0.04	0.25	0.22	-0.03
0.10	0.06	-0.04	0.25	0.20	-0.05
0.10	0.07	-0.03	0.25	0.23	-0.02
0.10	0.06	-0.04	0.25	0.22	-0.03
0.10	0.08	-0.02	0.25	0.19	-0.06
0.10	0.08	-0.02	0.5	0.39	-0.11
0.10	0.08	-0.02	0.5	0.45	-0.05
0.10	0.09	-0.01	0.5	0.39	-0.11
0.10	0.08	-0.02	0.5	0.45	-0.05
0.10	0.06	-0.04	0.5	0.38	-0.12
0.10	0.07	-0.03	0.05*	0.021	-0.029
0.25	0.22	-0.03	0.05*	0.021	-0.029
0.25	0.19	-0.06	0.10*	0.064	-0.036
0.25	0.18	-0.07	0.10*	0.059	-0.041
0.25	0.23	-0.02	0.15*	0.134	-0.016
0.25	0.21	-0.04	0.20*	0.16	-0.04
0.25	0.20	-0.05	0.20*	0.125	-0.075
0.25	0.19	-0.06	0.25*	0.13	-0.12
0.25	0.22	-0.03	0.25*	0.202	-0.048

\*Determinations carried out by one person.

noted that the same amount of lead was added to the specimen of urine. In addition, copper, iron, zinc, mercury, and bismuth were added in order to discover the effect upon the determination. The composite sample of urine gave a negative value for contained lead. It is apparent that bismuth and copper are substances which interfere most seriously with the method. The recovery values in Tables I, II and IV represent the composite efforts of six technicians employing the methods used in our laboratory. It is quite apparent that if there is an error in reporting the values, it is on the negative side; i.e., the values may be too low. The microburettes employed throughout this work have been carefully calibrated and corrections made. All solutions and reagents are regularly checked for contamination. The colorimetric procedure proved inadequate for our needs. The time involved in making up the various

standards for comparison was too great. We also felt that we could not obtain the same accuracy with this method as with the titrimetric determinations.

#### SUMMARY

The previous tables have indicated that the volumetric method for the detection of minute quantities of lead can be used in examining biologic specimens. The tables clearly indicate the extent and conditions to which this method may be employed. The method is applicable in detecting lead in amounts varying between 0.02 and above 0.50. The accuracy decreases as the larger amounts of lead are found. Tables I and II indicate the range of the method described.

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### FRACTIONAL URINE (DIABETIC) CHART\*

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DAVID W. KRAMER, M.D., PHILADELPHIA, PA.

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IN MANAGING a case of diabetes, daily supervision of the diet, urinary findings, insulin dosage and its administration, weight, blood chemistry, etc., is necessary. To facilitate matters, the Joslin diabetic chart was devised, thereby permitting one to obtain considerable information without turning over numerous treatment sheets and laboratory reports. Recently, the importance of frequent urinalysis has been stressed. While the twenty-four-hour urine specimen no doubt tells exactly how much glucose is being excreted, it does not, however, give all the information that is desired.

In certain cases, such as postoperative diabetics, patients with impending coma, or where severe forms of diabetes exist and require immediate standardization, there is a necessity for more frequent observations. The ideal method would be to have regular periodic analyses of the blood and urine. On occasions, there is difficulty either in obtaining the blood or facilities do not permit frequent blood tests for sugar. However, there should be no difficulty in arranging for urinalyses every two hours or whenever a specimen is obtainable. In this manner we may learn how the patient tolerates the meals, whether or not the insulin administered is sufficient to take care of the added load of carbohydrates, and, if there is a "spill over," where and when it occurs. The fractional method of urinalysis can be instituted at the patient's home or in the hospital. Nurses are taught how to test for sugar and are en-

\*From the Medical Department of Jefferson Medical College and the Diabetic Clinic of Jewish Hospital.

Received for publication, May 1, 1934.

TABLE I

## FRACTIONAL URINE (DIABETIC) CHART\*

Mr. S. C., aged seventy, height 5 feet 6 inches, and weight 133. Diabetic with gangrene of a toe and cellulitis of the foot. Operation under local anesthesia. Diabetic condition guided mainly by fractional urinalysis and occasional blood sugar estimations.

DATE	URINALYSES										DIET			INSULIN		BLOOD			WT.	R. P.	REMARKS					
	A.M.		P.M.		AMT.	SP. G.	SUGAR % GM.	ACET.	DIAC.	ALB. CAST	C.	P.	F. CAL.	DOSE / TIME	TOT.	SUG.	UREA	CO <sub>2</sub>				WASS.				
	7	9	12	2																			5	8	12	24
Feb. 24						4+	1500	1030	1.25	18.7	Pos.	Pos.	0	0	120	65	90	15	15	30	264			131	158/78	
25	2+	4+	2+	3+	+	1500	1032	2.5	37.5	Pos.	0	0	0	0	0	120	5	90	15	15	35					
26	4+	2+	2+	3+		1520	1021	0	0	0	0	0	0	0	0	20	5	15	5	45						
27	4+	2+	2+	2+	+	800	1026	0	0	0	0	0	0	0	0	20	5	15	5	45						
28	+	+	+	0	0	1300									0	20	10	20		50				131½		
Mar. 2	3+	2+	2+	2+	2+	2600	1020	0	0	0	0	0	0	0	0	120	65	80	20	10	20	50	160		132½	154/78
3	0	0	0	0	0	1400	1014	0	0	0	0	0	0	0	0	20	10	20		50						
7	0	0	+	0	0	2000	1020	0	0	0	0	0	0	0	0	20	10	20		50						
8	0	0	0	0	0	1500	1024	0	0	0	0	0	0	0	0	22	10	20		52						
11	0	0	0	0	0		1032	0	0	0	0	0	0	0	0	22	10	20		52						
12	0	0	0	0	0		1023	0	0	0	0	0	0	0	0	22	10	20		52	118			133		

\*Fractional urine (diabetic) charts are available and may be obtained from the Fehn Advertising Co., 212 Vine Street, Philadelphia, Pa.



couraged to make these examinations, not to send the specimens to the laboratory. The results are to be recorded promptly on the chart so that they are available whenever the physician-in-charge visits the patient.

It is not my intention to dilate upon fractional urinalysis, but merely to mention briefly its advantages and its practical application. To simplify matters and avoid the necessity of keeping an extra record, the author has arranged the fractional urine (diabetic) chart so that all the necessary information can be seen at a glance. There should be no difficulty in keeping these records. They are sufficiently complete for practical purposes.

These records will be found to be particularly useful in standardizing young diabetics, early severe cases, impending coma, postoperative diabetics, and in patients where there is difficulty in obtaining blood or where blood tests cannot be carried out.

Its advantages are:

1. Time saving, avoid the necessity of searching through many pages of the record.
2. It gives an opportunity to regulate the dosage of insulin.
3. It permits us to properly distribute the doses and to administer the insulin at periods where it will do the most good.

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## A RAPID METHOD FOR THE DEMONSTRATION OF NEGRI BODIES\*

JAMES R. DAWSON, JR., M.D., NASHVILLE, TENN.

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**B**ECAUSE of the familiarity of most pathologists with the various methods which have been described for the demonstration of Negri bodies in brain tissue no space will be devoted to a summary of the literature on this subject. So much work has been done and the methods have evolved so gradually that it would be impossible to acknowledge all of the contributions which have been responsible for the development of the procedure to be described. It is thought that this method is deserving of publication because of its simplicity and practical value.

The brain of the animal to be examined is taken out as soon after death as possible and several small segments of Ammon's horn are removed and placed in a Petri dish. These sections are cut so that they measure 3 or 4 mm. in thickness. The cut surface is of course perpendicular to the long axis of this structure. In removing these segments the tissue adjacent to the hippocampal fold should be trimmed off so that the horn alone is saved. Other portions of the brain of approximately the same thickness may be used also but in this laboratory Ammon's horn has been found most satisfactory because

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\*From the Department of Pathology, Vanderbilt University Medical School.  
Received for publication, July 6, 1934.

of the abundance of ganglion cells and the regularity with which these cells are involved in animals dead of street rabies.

One of these small segments of Ammon's horn is then placed, one of the cut ends down, on the smaller end of a new one-inch cork. Using a wooden applicator or a match, the superficial white matter, ependyma and other peripheral tissues are gently wiped downward and outward over the adjacent surface of the cork. This procedure attaches the tissue more firmly to the cork, pulls the white matter and ependyma downward and causes the gray matter to bulge upward.

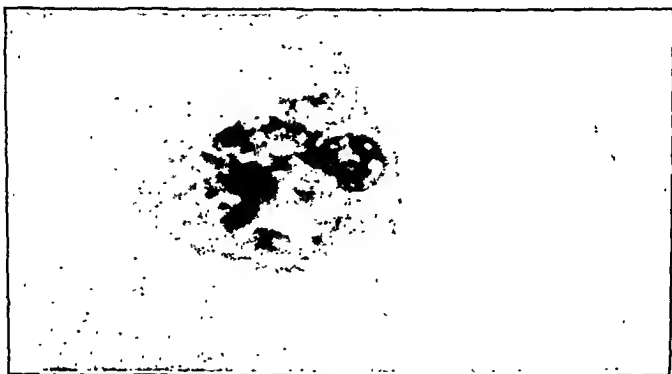


Fig. 1.—Ganglion cell with vacuolated Negri body.

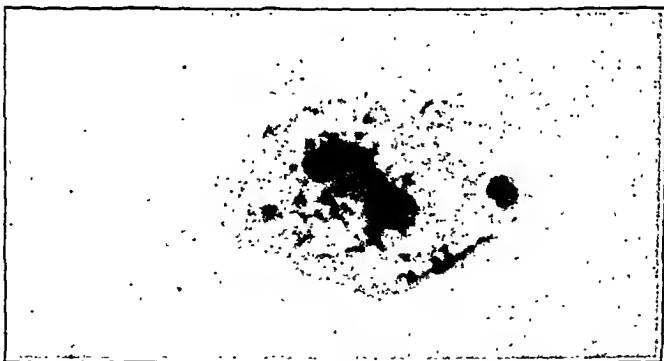


Fig. 2.—Ganglion cell showing nucleus, nucleolus, and at right Negri body.

A thoroughly cleaned glass slide held at one end by the thumb and forefinger is pressed gently against the segment of the horn and then raised. The slide should be held as nearly as possible at right angles to the segment of brain. The procedure is repeated 3 or 4 times on each slide starting at the end of the slide away from the fingers. This should be done rapidly so that the tissue does not dry. The slide is immersed quickly in absolute methyl alcohol where it is allowed to remain for five minutes or longer. Several slides are prepared in this fashion. It is necessary to increase the pressure slightly for each successive slide. It is advisable also to wipe the white matter down on the cork again if several slides are to be made. It is absolutely essential

that the slides be clean and entirely free from grease and dirt. If this precaution is not observed the preparations will be quite poor or worthless.

After fixation in alcohol the slides are rinsed in running water and stained with a 2 per cent aqueous solution of phloxine for two to five minutes. The excess stain is washed off in running water and the preparation is then stained in Loeffler's alkaline methylene blue for ten to twenty seconds. For the sake of convenience and safety the slide is handled with a pair of intestinal clamps. The slide is then decolorized in 80 per cent alcohol, dehydrated by passing through 95 per cent alcohol and two changes of absolute alcohol, cleared in xylol and mounted in balsam. In this laboratory wide mouth, glass stoppered bottles are used as containers for the alcohols and xylol. The slide held in the blades of the intestinal forceps is then passed from one to the other. This not only facilitates the manipulation of the slide but reduces the danger of contaminating the hands.

During this procedure the film will appear brilliant pink after staining with the phloxine. Then after staining in methylene blue it appears dark

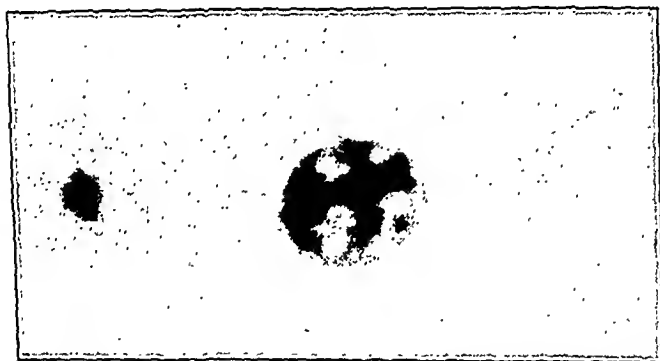


Fig. 3.—Higher power showing Negri body with inner structures in vacuoles.

blue or bluish black. The preparation should be left in the alcohol for several minutes, and should be decolorized until only the faintest tinge of blue and pink is seen when the slide is held against a white background. There is very little danger of carrying the decolorization too far. The longer they are allowed to remain in alcohol the more brilliant the blue becomes. For this reason it is advisable to overstain the film with phloxine and methylene blue so that the process of decolorization and dehydration will not have to be rushed. If the staining times indicated are observed the films will be slightly overstained.

Examination of the finished slide with the naked eye against a white background will reveal a thin bluish line which is composed of ganglion cells. There is of course quite a bit of distortion of normal relationships but one will be surprised to see how well they are preserved. When examined under the microscope the pyramidal cells are easily distinguishable from the cells of the stratum oriens and glial cells. In satisfactorily prepared films the cytoplasm of the pyramidal cells and their processes are well preserved. They

stain a clear bright blue. The nucleus is also well preserved. Its chromatic material stains pale blue. The nucleoli are large, dense and bluish black. If the decolorization is incomplete these portions stain purple rather than blue and appear muddy rather than clear. It is certain that the cells are more easily studied in those preparations in which the cytoplasm is a bright, clear blue. The degree of decolorization of those elements which take the phloxine is of considerable importance. It is possible to remove all the stain from the red cells and still leave the Negri bodies deeply stained. However, it is also possible to destain the Negri bodies completely. Before one has become entirely familiar with the method, it is best to decolorize the films until the red blood cells stain pale pink. In such preparations the Negri bodies are easily distinguishable.

In these preparations the smaller Negri bodies stain brilliant red and appear homogeneous and refractile. The larger bodies have a brownish tint. Within them one sees well-defined pale bluish vacuoles and dense blue black granules. The reddish brown matrix of the larger bodies also appears refractile. In satisfactory preparations it is quite easy to show that these bodies are definitely intraeytoplasmic. Of course in most preparations many of the Negri bodies are not in the cytoplasm. However, even in such cases they can be differentiated or identified with some certainty.

With a little practice it is possible to prepare and stain the films so that the red cells are hardly visible while the Negri bodies stain bright red or reddish brown. Even under low power they can be recognized with some certainty because of their sharpness and refractivity. The stain which the Negri bodies take is of course dependent upon the length of staining and degree of decolorization. If they are not slightly overstained and allowed to remain in the alcohols for quite a while it may be impossible to make out their inner structure. Even then little difficulty is encountered in identifying them. Of course for careful study of the bodies it is important that their inner structures be properly stained and differentiated.

In this laboratory it has been observed that if Negri bodies can be found in Zenker's fixed paraffin sections stained by the carbolaniline-fuchsine-methylene blue method of Goodpasture or any other stains they can also be observed in preparations of the type described in this paper.

In some instances it has been found that the Negri bodies are more obvious in the film preparation than in fixed sections. It is certainly true that they are more easily demonstrable by this method than they are in rapid paraffin sections of fixed brain tissue.

This method is not suggested as a substitute for the routine section methods of fixation and staining. At the time tissues are taken for film preparations, blocks are also fixed in Zenker's solution and run through so that in case no Negri bodies are found in the former they may be searched for in the latter. It is believed that Negri bodies can be demonstrated in film preparations of Ammon's horn of animals dying of street rabies if it is possible to demonstrate them by any other method. The method just described, includ-

ing the microscopic examination, may be completed in twenty-five minutes from the time the incision is made in the scalp to remove the brain until the diagnosis is made.

#### CONCLUSIONS

1. A rapid and satisfactory modification of the impression method for diagnosis of rabies is described, with a staining technic employing phloxine.

2. It is judged that this method is practically as accurate as the routine tissue section methods.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

## GLUCOSE TOLERANCE, In Children and Adolescents, John, H. J. *Endocrinology* 18: 75, 1934.

This study represents an analysis of the results of glucose tolerance tests in 192 children. The results have been compared with those obtained in a study of approximately 1,500 adults. Normal curves were obtained in 82 per cent of the children as contrasted with only 62 per cent in the adult group. There was a low incidence of diabetic curves in the first four decades, after which the rise was more abrupt with the peak in the seventh decade.

There was no appreciable difference between the normal fasting blood sugar level in children and in young adults; the values fluctuated between 60 and 120 mg. per cent, although most fell between 71 and 110 mg. per cent.

Among the patients was a group of 25 children in whom diabetes developed within four to thirty days following severe infections. The data from these indicated that infections may produce temporarily decreased glucose tolerance and also may play a definite and considerable part in the production of permanent diabetes in children.

In 52 children with glycosuria, 33 per cent showed a diabetic type of glucose tolerance curve. Similarly, in a series of 337 adults with glycosuria, 125, or 37 per cent, showed a diabetic type of curve.

Only three of 17 children with hyperthyroidism showed a diabetic type of curve. This contrasts with the finding of 63.5 per cent diabetic curves in a group of 239 adults with hyperthyroidism.

Of 14 obese children, only two showed decreased sugar tolerance in striking contrast to the findings in 172 obese adults, 113 or 65.6 per cent of whom showed a diabetic type of curve.

Some workers tend to minimize the importance of decreased sugar tolerance in children, regarding it as the result of various factors, but this finding should never be disregarded, for only by continued study of these children will the true significance of decreased sugar tolerance be revealed.

## A-DINITROPHENOL, Detection and Estimation of, A New Drug for the Treatment of Obesity, Bollinger, A. *Med. J. Australia* 1: 367, 1934.

Despite the reports illustrating the toxic and even fatal effects of a-dinitrophenol, its recent use in the treatment of obesity will, without doubt, be followed, indeed, has already been followed, by its indiscriminate use by the laity without medical supervision. It may also be expected that, openly or secretly, it will soon form part of the proprietary products sold for the self-treatment of obesity.

The tests devised and described below for its detection in urine and blood are applicable to cases of suspected poisoning with this drug:

The tests are based upon the fact that 2:4 dinitrophenol forms very readily with methylene blue an addition compound similar to that formed by picric acid. By simply adding a solution of methylene blue to a solution of dinitrophenol methylene blue, dinitrophenolate is obtained, which crystallizes in fine bronze-colored needles. This compound is sparingly soluble in most solvents to form a green solution. Its solubility in chloroform is greater, but considerably less than that of the corresponding picrate. The practical application of this reaction in the detection of a-dinitrophenol is as follows: The

acidified solution is extracted with chloroform and to the neutralized chloroform extract a dilute solution of methylene blue is added. If dinitrophenol or a similar compound is present, the chloroform extract will become green.

The methods follow:

*Urine.*—In a separating funnel a volume of urine (20 c.c., if available) is acidified with one-tenth of a volume of 70 per cent sulphuric acid. It is then extracted by gentle shaking for about three minutes with half its volume again of chloroform. If possible, permanent emulsions have to be avoided. However, if after some standing sufficient chloroform does not separate out cleanly, the chloroform has to be separated out by centrifugation. Then about 10 c.c. of the chloroform extract are transferred to a test tube containing a gramme or so of calcium carbonate. The contents are mixed well and 0.0001 N methylene blue, chemically pure (about 0.004 per cent) is added in very small drops.

The mixture is well shaken after every drop till the first change of color of the contents occurs. It is then filtered through a dry filter into another test tube, an equal amount of distilled water is added, it is shaken well again and the water is removed. If the chloroform shows a distinct green color which cannot be extracted by further washings with water, *o*-dinitrophenol is present in the urine. If there is any doubt, some of the chloroform extract may be concentrated by evaporation in order to intensify the color.

If dealing with amounts of dinitrophenol up to one milligram per centum in urine, the application of the precautions mentioned will be necessary, because some substance present in normal urine in small amounts and extracted by chloroform seems to form with an excess of methylene blue a bluish compound, which may easily be misleading. Therefore, for the detection an excess of methylene blue has to be avoided. But after the presence of dinitrophenol has been established by the typical greenish color of the chloroform extract, more methylene blue may be added either to get an approximate idea of the amount of the drug present or to perform an actual quantitative estimation by the technic which will be described below. Doubtful results with regard to the presence or absence of the drug may be compared with those obtained with urine treated similarly, which are known to contain no dinitrophenol. With amounts above one milligram per centum the results are beyond doubt.

*Blood.*—Serum acidified with a few drops of sulphuric acid is thoroughly shaken with an equal amount of chloroform. Then the mixture is centrifuged and the chloroform extract is pipetted off and filtered through a dry filter into a test tube containing some calcium carbonate. Then 0.0001 N methylene blue is added in small drops till the mixture begins to change color. After filtering again, the presence of dinitrophenol is indicated by a green tint of the chloroform extract.

*Solutions.*—A known amount is dissolved in 5 per cent sodium hydroxide. After the solution is acidified with 70 per cent sulphuric acid, the dinitrophenol is extracted with several lots of chloroform. The combined chloroform extracts are treated with calcium carbonate, filtered and made up to a known amount. An aliquot part of the chloroform extract is transferred to a separating funnel and 0.0001 N methylene blue is added from a buret. The methylene blue combines with the dinitrophenol to the chloroform soluble methylene blue dinitrophenolate. Therefore, on extraction the watery layer originally containing the methylene blue turns yellow, while the chloroform takes on a green color. The end-point is reached when the watery layer becomes colorless. For practical reasons the appearance of the first tint of blue which cannot be extracted with fresh chloroform is regarded as the end-point. In other words, when one has reached a definite end-point, the dark green chloroform must be discarded and the watery layer must be extracted with fresh chloroform. It will then usually be found that the end-point has not yet been reached and more methylene blue has to be added, followed by further extraction with fresh chloroform. As already mentioned, the solubility of the methylene blue dinitrophenolate in chloroform is comparatively small and it is practicable only for amounts up to about 10 mg. of dinitrophenol to be determined by this procedure. If dealing with larger amounts, it is recommended to precipitate all of the dinitrophenol with a small excess of 0.01 N methylene blue and to determine the excess of methylene blue as

described in a previous publication. (The Volumetric Determination of Methyleno Blue and Pieric Acid, Bollinger, A. *Præ. Ray. Soc. New South Wales*, 67: 240, 1933. Not available for abstract.)

As already mentioned, the test described is not specific for 2:4 dinitrophenol. A similar compound is given by 2:4:6 trinitrophenol (pieric acid), and this could be differentiated from the dinitro compound by the considerably greater solubility in chloroform. In general it seems that all polynitro derivatives of phenol and naphthol with a nitro group in ortho-position to the hydroxyl group form with methylene blue an addition product which dissolves in chloroform with a green color.

**POLIOMYELITIS, Acute, Therapy by Blood Transfusions From Immune Donors, Sherman, I. *Am. J. Dis. Child.* 47: 533, 1934.**

Seventy-one cases of acute poliomyelitis in which transfusions from convalescent donors were given are reported.

The results obtained are compared with those observed after injection of convalescent serum and those reported in other statistics during the same epidemic.

Transfusions from immune donors decreases the mortality, and if given during the preparalytic stage decreases the incidence of paralysis. It favorably influences the course of the infectious symptoms and is conducive to an almost critical improvement of the patient in the majority of cases.

Transfusion from immune donors appears to be the method of choice in the treatment of acute poliomyelitis.

It is suggested that health authorities or a central bureau provide for registration of convalescents in order to have donors available at the time of a new epidemic.

**AGGLUTININS, In Mother's Blood, Baby's Blood, Mother's Milk, and Placental Blood, Toomey, J. A. *Am. J. Dis. Child.* 47: 521, 1934.**

The placental blood of human infants contained agglutinins against the enteric group of organisms. In the specimens examined the agglutination titer usually did not go beyond 1:40, but occasionally it reached 1:80.

The mother's first milk and the specimen of blood taken from the infant ten days after birth showed a marked and about equal increase in agglutinins as compared with the placental blood from the same patient.

In the specimens examined, the mother's serum always contained massive agglutinins against the enteric group of organisms.

In the human being, a small amount of agglutinins against enteric organisms passes through the placenta. The later acquisition of an increased amount as shown by the agglutination titer is probably brought about by absorption from ingested milk.

**EDEMA, Concentration of Serum Protein in Different Types of, Hand, H. M. *Arch. Int. Med.* 54: 215, 1934.**

Normally plasma contains from 6.2 to 8 gm. of protein per hundred cubic centimeters, of which from 3.6 to 5 gm. is albumin and from 2 to 3.5 gm. is globulin. The albumin globulin quotient is from 1.2 to 2.2.

Low levels of serum proteins and resulting edema may be found in a variety of clinical conditions and may be due to a number of causes such as: (1) insufficient intake of protein due to an inadequate diet; (2) excessive loss of protein due to proteinuria, diarrhea or hemorrhage; (3) excessive metabolic wastage or destruction of protein due to chronic infections or cachectic states; (4) inadequate formation of proteins or decreased assimilation. The various clinical conditions in which low serum protein levels and edema appear owing to one of the aforementioned causes are discussed.

As the serum protein levels fall, edema usually appears when the protein total reaches 5.5, 0.3 gm. per hundred c.c. or when the albumin reaches 2.5, 0.2 gm. These are



frequently called the critical levels since they are the levels at which edema usually appears and at which the hydropigenous tendency is most easily affected by other factors such as the intake of sodium chloride.

Deficits of serum protein fall principally on the albumin fraction. The globulin fraction may be normal, decreased, increased slightly as a compensatory effort or increased markedly, especially when infection is present.

The ratio of albumin to globulin is, in itself, probably of little or no importance, since an inversion of the ratio due to an increase in the serum globulin has a significance entirely different from that of an inversion due to a decrease in the serum albumin.

The intake of sodium chloride and the electrolytic pattern have an important influence in modifying the edema associated with low levels of the serum proteins.

Low levels of serum proteins, with resulting edema, are best prevented by adequate ingestion of protein. The best treatment is by a diet high in protein, restriction of sodium chloride intake and at times use of acidifying diuretics, transfusions of blood or plasma and intravenous administration of aecacia.

**GONOCOCCI, New Medium for Isolation of "In Vitro,"** del Castillo, H., and Herraiz, L. Arch. de med., cir. y espec. 37: 710, 1934.

There are 500 gm. of beef, deprived of aponeurosis and fat, and 20 gm. of eaked brewers' yeast in 1,000 gm. of water left in maceration for fifteen hours. These ingredients are then boiled for fifteen minutes and filtered, enough water being added to make the original volume. Then 20 gm. of Witte's peptone and 0.5 gm. of potassium chlorate are added. The pH is then fixed at 7.6 and the preparation boiled again, but only for a short time to precipitate the phosphates, and then filtered again. Agar-agar is then added in a proportion of 3.5 per cent; that is, 3.5 parts of agar-agar to 96.5 parts of the preparation. The preparation is then sterilized for three successive days for thirty minutes at a time in high pressure steam. After it is melted at a temperature of 45° C., rabbit's or horse's blood is added in the proportion of 20 per cent; that is, 20 parts of blood to 80 parts of the preparation. The medium is then ready to be placed in separate sterile receptacles for use.

**PREGNANCY, Histologic Diagnosis of Age of From Percentage of Erythrocytes in Chorionic Capillaries,** Ryerson, C. S., and Lanes, S. Arch. Path. 17: 648, 1934.

Specimens are fixed for sixteen hours in 10 per cent formaldehyde, dehydrated in alcohol and chloroform, and paraffin sections (8 microns) stained with H and E.

The percentage ratio of nucleated and nonnucleated red cells is determined in from 100 to 200 red cells in the chorionic capillaries.

If all the chorionic corpuscles are nucleated, the pregnancy is probably not older than two months; if more than 1 per cent are nucleated, the age is less than three months; less than 1 per cent of nucleated reds indicates pregnancy more than three months old.

**POLIOMYELITIS, Experimental and Theoretical Basis for Serum Therapy: A Review,** Harmon, P. H. Am. J. Dis. Child. 47: 1179, 1934.

An attempt has been made in this review to compile and present in one place the experiences of numerous investigators who have treated acute epidemic poliomyelitis, emphasizing results that have been obtained with various types of serums. Reports of 4,400 cases are analyzed in which the patients were treated with serums and compared with these were 2,660 cases in which no such treatment was given, the two series occurring coincidentally. Of the treated patients, 2,637 were seen early in the disease, the majority being in the preparalytic stage. It has been pointed out again that evaluation of the influence of a therapeutic agent in this disease especially must be a detailed and complex analysis, which will include variable factors that have been largely ignored until the past few years. The presentation of results should be standardized and extended to include even the rate and extent of recovery of muscular function in the reparative stage, since

it is not inconceivable that serum or other agents may have an indirect influence even at such a remote date. Harmon proposes a standard scheme for the estimation of results and has attempted to transfer partially the results of others to the terms of this system. The limitations of this presentation are those imposed by the data available.

Since there is about equal experimental evidence in favor of all four types of serum that have been used, namely, the antistreptococcus horse serum (Rosenow and Nuzum and Willy), the antiviral animal serums (Pettit and others) and both convalescent and "normal" human serum, only the results that have been actually obtained in clinical trial of these serums can settle the question of the supremacy of one type.

Early diagnosis and treatment have been held as imperative for success in the serum treatment of this disease. The uncertainty of the course of the earliest type of poliomyelitis, the preparalytic stage, has been responsible for the apparently brilliant results when treatment has been applied in this stage. The explanation for apparently favorable results when treatment is applied in the preparalytic stage is probably the fact that many cases regarded as preparalytic are in reality nonparalytic poliomyelitis. The author has collected the statistics on the fate of 531 untreated patients with preparalytic poliomyelitis; these show that 380 or 71.5 per cent never had paralysis at any time. The outcome in patients treated in the preparalytic stage does not differ from the average for untreated patients. Recent therapeutic experiments in which convalescent serum was given to alternate patients by Kramer and his associates and by Park support the same contention, showing no difference in the average outcome in treated and untreated patients.

It has again been pointed out that the nature of the epidemic, the point on the epidemic curve when treatment was applied, the age of the patient and the type and degree of orthopedic after-care are all factors of extreme importance in estimating the effect of any program of treatment. In the past these factors have been frequently ignored.

Notwithstanding the total failure of statistical presentations to favor certain types of serums, clinical observations that have been almost universally made of rapid symptomatic response to the administration of serum by an immediate drop in temperature and marked improvement in symptoms cannot be totally disregarded. All other forms of therapy in this disease have been a signal failure, with the possible exception of spinal drainage. Chemotherapy, medicinal therapy and artificial fever have all been tried without benefit. More data are needed before it can be said conclusively that serum of any of the four types is totally without value. There appears to be enough evidence from clinical observation to warrant the continued use of serums in early stages of acute poliomyelitis.

**LEUKOCYTOSIS, After Parenteral Injection of Liver Extract, Meyer, O. O., Middleton, W. S., and Thewlis, E. M. Am. J. M. Sc. 188: 49, 1934.**

The parenteral administration of concentrated liver extract (Lederle) was ordinarily attended by an early fall in the white cell count and a constant subsequent leucocytosis with increases in total and relative numbers of neutrophils in 4 normal and 3 abnormal individuals.

The maximal leucocyte count was usually attained 3 hours after the intravenous injection of the extract and 5 hours or later after the intramuscular injection. After the intramuscular injection the rise, though less prompt, was better sustained.

In a patient with chronic leucopenia there was an increase in leucocytes to a maximum of 73 per cent above the control period level.

In a patient with Banti's disease with leucopenia, leucocytosis was produced repeatedly by the parenteral injection of liver extract both before and after splenectomy. Also, a shift to the left in the Schilling count occurred on the occasion of an intramuscular injection before splenectomy. This was two days after the intravenous administration of the same extract.

Leucocytosis also occurred before and after splenectomy in a patient with familial hemolytic icterus.

Since leucocytosis can be produced by the intramuscular injection of this concentrated liver extract after splenectomy, it would appear that this substance does not produce the increase in the leucocytes by inducing splenic contraction, but possibly by a stimulation of the bone marrow, directly or indirectly.

The administration of liver extract to induce an elevation in the white count, at least temporarily, is logical. Its efficacy and limitations will be established by a thorough clinical trial.

**LIVER FUNCTION, Cinchophen Oxidation Test of, in Pulmonary Tuberculosis, O'Connor, J. B., Young, H., Steidl, J., and Heise, F. H. Am. J. M. Sc. 188: 81, 1934.**

Briefly, the cinchophen oxidation test consists in feeding the patient 0.45 gm. of cinchophen and determining colorimetrically the output of oxycinchophen in the urine. According to Lichtman 100 mg. or less of oxycinchophen are excreted in the urine of normal subjects. When an amount in excess of 100 mg. is found in the urine, liver insufficiency is considered to be present, higher figures indicating greater insufficiency.

Hepatic insufficiency, as indicated by the cinchophen oxidation test of hepatic cell function, occurred in more than 90 per cent of a group of consecutive unselected patients with pulmonary tuberculosis admitted to Trudeau Sanatorium.

**TUBERCULOSIS, The Frequency of Tuberculous Bacillemia by Lowenstein's Method, Peterson, W. F., and Lederman, I. H. Am. Rev. Tuberc. 30: 103, 1934.**

The authors summarize the developments of methods for the demonstration of tubercle bacilli in the circulating blood and report their own experience with Lowenstein's method:

In a series of 74 cases of pulmonary tuberculosis attempts to demonstrate tubercle bacilli in the blood stream by cultural methods were completely negative.

The results of most workers make it apparent that the use of Lowenstein's method is seriously open to question as to efficiency for use in the demonstration of a tuberculous bacillemia.

**TUBERCULOSIS, Tuberculous Bacilluria, Montgomery, L. G., and Allen, R. B. Am. Rev. Tuberc. 30: 92, 1934.**

A series of experiments was performed for the purpose of demonstrating if it were possible to bring about passage of acid-fast bacteria through the normal kidney.

Two experiments were carried out first: in one, guinea pigs were given intravenous injections of a heavy suspension of *Mycobacterium avium*, and in the other, rabbits were similarly given injections of a heavy suspension of *Mycobacterium phlei*. The urine of these animals was collected by catheterization, examined microscopically, and cultured by appropriate means for the demonstration of acid-fast bacteria. From two guinea pigs and one rabbit cultures of organisms similar to those injected were obtained from the urine.

Two further experiments were performed, similar to the first two, except that the number of bacteria was doubled in an attempt to obtain more striking results. The results were negative in the case of the rabbits, but 82 per cent of the cultures made from the guinea pigs were productive of bacterial growth.

Finally, a fifth experiment was carried out, similar to the two experiments on guinea pigs except that the urine was collected by aspiration of the bladder at necropsy. A further change was made by introducing a group of guinea pigs in which *Mycobacterium phlei* was used in place of *Mycobacterium avium*. Positive cultures were not obtained in this experiment. Consequently it was considered that the positive results of the first experiments were due to contamination of the urine by blood elements which had been liberated through abrasions caused by the introduction of the catheter.

The authors conclude, therefore, that the normal kidney of the rabbit and the guinea pig is not permeable to the acid-fast organisms used in this investigation, even in the presence of marked and continued bacillemia.

**TUBERCULOSIS, The Lymphocyte Reaction in,** Wiseman, B. K., and Doan, C. A. *Am. Rev. Tuberc.* 30: 33, 1934.

In this study an analysis of the qualitative changes occurring in the lymphocyte, which the authors believe reflect directly the functional state of the lymphopoietic centers, has been applied to disease. For various reasons tuberculosis, in its experimental and clinical phases, furnished the best initial test of this hypothesis.

In the experimental studies it has been possible to follow in a single animal the entire natural history of the disease. A varying efficiency in the response of the lymphatic tissues from time to time was reflected by the changes in the circulating lymphocytes, which in turn seemed to represent a very fair measure of the adequacy of the general immunological reactions of the animal. A judicious interpretation of both quantitative and qualitative data is essential to the fullest understanding.

A careful selection of patients representative of the various stages of the disease has provided a basis for a similar analysis of the lymphatic reaction in clinical tuberculosis. In these studies the lymphatic response has been far more sensitive, and has preceded other evidence of a changed status in the disease more consistently than any other criterion thus far used. It may thus be of distinct prognostic significance.

Additional evidence is furnished in these studies of the direct part played by the lymphocyte in the cellular defense of the body against tuberculosis.

It is desirable to have as many criteria as possible in the analysis of any given tuberculous case, and even so the frequency of unexpected complications always renders prognosis hazardous. In a comparative study of a number of different determinations upon which reliance has been placed in the past, something more of their relative merits has been ascertained. More specifically, the following of the hemoglobin level, a study of the quality, "R" versus "S" colonies, rather than the quantity of the acid-fast organisms present in the sputum from time to time, and the determination of the titer of the phosphatide-precipitating capacity of the patient's blood serum, have yielded much valuable information in this study.

Emphasis, finally, is to be placed upon repeated examinations in the same patient, with exquisite technical precision rather than a single casual observation, if the full significance of any procedure is to be realized. Tuberculosis ordinarily runs a long chronic course with exacerbation and remission and with gradual trends rather than a stationary equilibrium. Therefore, a better understanding of the inherent resistance forces, and more particularly their full conservation by appropriate adjuvant measures, in the individual patient, is essential at the present time for the intelligent management of the disease.

**JAUNDICE, Catarrhal, Residual Hepatic Damage in, as Determined by the Bilirubin Excretion Test.** *Arch. Int. Med.* 53: 809, 1934.

Eleven patients who had had catarrhal jaundice within from three months to eighteen years prior to the investigation were studied by means of the bilirubin excretion test for evidence of residual hepatic damage. It must be emphasized that the original attack was free from such complications as acute yellow atrophy of the liver. Nine of the eleven patients showed degrees of retention of the injected pigment that varied from 10.7 to 50 per cent. Six of the nine patients had had catarrhal jaundice within from three to eighteen years before the investigation.

The degree of residual hepatic damage apparently bears no relationship to the severity of the attack. In view of the response to the test in these cases it is concluded that catarrhal jaundice is not innocuous as is commonly believed, since in a good many instances an impairment of hepatic function occurs which is permanent.

**METHYLENE BLUE, Intravenous Injection of, in Man With Reference to Its Toxic Symptoms and Effect on the Electrocardiogram,** Nadler, J. E., Green, H., and Rosenbaum, A. *Am. J. M. Sc.* 188: 15, 1934.

These observations indicate that methylene blue, under the conditions of this study, has two actions. The first of these is the oxidation of hemoglobin to methemoglobin.

The amount of methemoglobin found immediately following the injection of the average therapeutic dose is small.

The second is that this drug, used intravenously, excites the individual and by its rapid elimination into the stomach and urine produces transitory gastrointestinal and urinary irritation. The most frequent toxic symptoms observed were restlessness, paresthesias, a sense of "burning" in the mouth and stomach, a pain in the chest and strangury. These manifestations usually subsided in twenty-four to forty-eight hours. Leakage of a small amount of methylene blue about the vein gives rise to a very painful infiltration.

Electrocardiographic studies show that methylene blue produces a reduction in the height or even reversal of the T-wave frequently with lowering of the R-wave. This suggests depression of the ventricular musculature.

The amount of methemoglobin found and the subsequent decrease in hemoglobin is not of sufficient magnitude to account for the clinical picture described on the basis of anoxemia.

The authors therefore wish to point out that the indiscriminate use of methylene blue may produce unpleasant results and be dangerous to the patient.

**HYPERTENSION, Malignant, the Histologic Changes in the Kidneys, Cain, E. F.** Arch. Int. Med. 53: 832, 1934.

In a group of cases of malignant hypertension the findings on examination of microscopic sections consisted in diffuse changes involving glomeruli, tubules, arterioles, arteries, and interstitial tissue. The most prominent changes occurred in the arterioles; they consisted in extreme narrowing of the lumen, apparent increase in the numbers of endothelial cells, subendothelial fatty and hyaline degeneration, apparent thickening of the tunica media and an increased amount of connective tissue, chiefly in the tunica adventitia. The ratios of the wall to the lumen of the renal arterioles were markedly reduced. The kidneys were not markedly or uniformly decreased in size.

**WATER ANALYSIS, Comparison of MacConkey's Bile-Salt Broth and Dominick-Lauter Broth in Routine, Raghavachari, T. N. S., and Iyer, P. V. S.** Indian J. M. Res. 31: 735, 1934.

Parallel tests on 72 samples of water, from different sources, have been carried out using MacConkey's bile salt lactose broth and Dominick-Lauter methylene blue bromocresol-purple broth respectively as the enrichment and selective media. Comparison indicates that the MacConkey broth test is more sensitive and reliable.

Both as a selective medium for the presumptive positive test for coli, and as an enrichment medium to be followed up by subculture for the study of discrete colonies, and classification into coli, aerogenes and intermediates, MacConkey's broth (1.5 per cent bile) retains its position as the most satisfactory medium in water bacteriology.

**GRANULOMA INGUINALE, Sensitization and Antibody Production in, Pandalai, N. G., and Nair, V. G.** Indian J. M. Res. 31: 731, 1934.

Employing extracts of tissue obtained from the ulcers of granuloma genitoinguinale the evidence for the occurrence of allergic sensitization of the cells was not unfavorable in 25 cases while that for the presence of any specific complement-fixing antibody in the blood was negative in 11 cases.

# The Journal of Laboratory and Clinical Medicine

VOL. 20

MARCH, 1935

No. 6

Editor: WARREN T. VAUGHAN, M.D.

Richmond, Va.

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CLIFFORD J. BARBORRA, M.D.	CHICAGO

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## EDITORIAL

### Dissertation Upon Life in the Laboratory

(After—quite a long way after—Ogden Nash.)

It seems to me that it would not be at all surprising if pathologists were to develop, if such a thing were possible, a number of cerebral calluses

When you consider how much of their time is spent in the consideration of somewhat complicated analyses.

In fact, it's more or less of an oddity

That they don't as a rule dismiss the visitor to the lab. with simply a nod at he  
Instead of having to enter upon a long drawn out and sometimes apparently endless discussion,

Which, strangely enough, seldom leads to signs of cerebral concussion,

About why, in this particular case which has, of course, been very well worked up clinically—(sez you!)—and is without doubt a classical example of whatyoumaycallitis, the laboratory findings are so much at variance with the clinical diagnosis.

So that sometimes the pathologist is led to wonder (silently) if the clinician may not be suffering from a slight touch of intellectual astereognosis; While at the same time the clinician is thinking that the pathologist, who otherwise might have as much personal charm as Aspasia, Might not also have some sort of low mental visibility which, if one were talking about his walk instead of his talk, might be likened to astasia-abasia.

Yes, indeed, in a manner of spoken,  
It's a great life—if you don't weaken!

So it's no wonder if pathologists often have a somewhat harassed appearance like one who is confronted with a perpetual riddle,

Or as a man might look if he spent his life on a sort of an intellectual red-hot griddle.

It would not hurt to expend a little sympathy upon the pathologist for many and varied, indeed, are often his woes.

For one thing, it often seems as if the pile of requisitions never gets any smaller but always grows.

Then there's the nurse, for example, who thinks it is a good idea to order everything "STAT!"

So that she will be sure to have the report when the doctor comes in—even if that won't be for a week from next Thursday—and then he will be much impressed with the fact that she is always on the job and super-efficient, at that.

Not to mention the nurse, usually a graduate on private duty, who rushes in at 1 P.M. to know what the result was in the blood culture taken at 11 o'clock that same morning,

And thereby causes the sudden addition to the language of some very remarkable phrases which, however, all die a-borning.

But, come to think of it, neither of these has anything on the one who tips off the technician that, regardless of what the doctor calls it, *she* thinks it's pneumonia,

And, what's more, she has no intention of killing herself on the case for there's no sense in letting them think they own ya!

Oh, yes! The Resident did a blood count during the night and busted a pipette and three of the special cover-glasses for the blood counting slide—

Which really ought to be justification for taking it out of his hide.

But what's the use for it's quite obvious that he has no intention of ever being annoyed with any of these minor matters

But intends, when he goes into practice to leave such things to his hoi polloi fraters,

For, if one can judge from his manner the fact is

He intends to devote himself entirely to splenectomies or some other highly specialized practice.

Which may account for his not getting much out of his laboratory service.

Which he romps through more or less like a bull in a china shop, which is apt to make the pathologist somewhat nervous.

Dear me! And what can you do with the bottle of urine without any name on it or the smear with no requisition to tell you what to stain it for, or the small piece of tissue in alcohol labeled "from Mrs. Blockus,"

Or the man who comes in from the outside with something dried out on a small piece of cotton and wants you to make a smear for the gonococcus?

Of course, it's a great relief that the Resident who was sent up by the Superintendent to have a blood count because he looks pale and she *knows* he has an anemia,

Turns out to have a hemoglobin and red cell count just a little short of polycythemia.

Which gives you the strength to refrain from administering a couple of Kayos To the fellow who always wants to know why we can't do it here the way they do it at the Mayos;

And also to the man who wants the autopsy held over until well after his office hours so he can be present because he is *exceedingly interested in the case*, And then, after arriving even later than you expected, runs off as if he were in a race.

And—oh, well! As has already been said in a somewhat low manner of spoken—

It sure is a great life—if you don't weaken!

—R. A. K.



# *The Journal of Laboratory and Clinical Medicine*

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VOL. 20

APRIL, 1935

No. 7

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## *CLINICAL AND EXPERIMENTAL*

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### THE CHOLESTEROL CONTENT OF THE PLASMA IN ARTHRITIS\*

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EDWARD F. HARTUNG, M.D., AND MAURICE BRUGER,† M.D., NEW YORK, N. Y.

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THE extensive literature on blood cholesterol contains few records of studies on cholesterol metabolism in arthritis. Carbohydrate, protein and mineral metabolism have been investigated in detail, the general assumption being that fat (and sterol) metabolism in such cases is normal. Pemberton<sup>1</sup> observed that many patients with arthritis showed a delayed removal of glucose from the blood following its ingestion, and that a low caloric diet had a beneficial influence on the clinical course of the disease; these findings, he believed, indicated that these patients might show some disturbance in fat metabolism. Pemberton and Foster<sup>2</sup> determined the cholesterol and fat content of the whole blood and of plasma in fourteen patients with arthritis and found normal values. The plasma cholesterol in their cases ranged from 68 to 137 mg. per 100 c.c., which in the light of our present knowledge of the normal range for plasma cholesterol, may be considered as definitely low. Gorham and Myers<sup>3</sup> studied the cholesterol content of the blood in five cases of arthritis and found normal values. Bruger and Poin-dexter<sup>4</sup> reported observations on eleven cases, and they stated that, in general though not consistently, rheumatoid or gonococcal arthritis was associated with hypocholesterolemia and osteoarthritis with hypercholesterolemia.

These latter observations prompted a detailed study of the blood cholesterol in a large number of arthritides. The present investigation includes a study and statistical analysis of the cholesterol partition in the plasma in 92 cases of

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\*From the Department of Medicine, New York Post-Graduate Medical School and Hospital. Received for publication, July 30, 1934.

†Aided by a grant from the Josiah Macy Jr. Foundation.  
†Oliver Rea Fellow in Medicine.

arthritis and a correlation between the sedimentation rate and the cholesterol in a large number of these patients.

### MATERIAL AND METHODS

The subjects for this study were obtained from the Arthritis Clinic of the New York Post-Graduate Hospital. Only typical cases of rheumatoid or osteoarthritis were selected, the accepted criteria for their differentiation being carefully observed. In all cases, the duration of the disease varied from three months to ten years.

TABLE I\*

VARIATIONS OF THE FREE AND ESTER CHOLESTEROL AND OF THE SEDIMENTATION RATE IN RHEUMATOID AND OSTEOARTHRITIS

CASE NUMBERS	AGE RANGE YR.	SEX		PLASMA CHOLESTEROL RANGE			SEDIMENTA- TION RATE RANGE MM. PER HOUR
		MALE	FE- MALE	TOTAL MG. PER 100 C.C.	ESTERS MG. PER 100 C.C.	ESTERS PER CENT OF TOTAL	
<i>Rheumatoid Arthritis</i>							
1-10	17-55	5	5	100.0-153.8	56.4-107.1	48.5-76.3	13-46
11-20	27-55	3	7	153.8-180.3	74.1-137.9	46.6-76.8	6-47
21-30	21-55	3	7	182.0-226.0	105.4-142.0	51.7-72.0	8-41
31-33	32-55	1	2	230.0-302.5	122.5-178.5	51.0-59.0	14-21
Average	39			175.2	106.8	60.9	25
<i>Osteoarthritis</i>							
1-10	29-60	0	10	150.0-192.3	85.6-127.5	45.9-72.6	5-11
11-20	39-64	3	7	193.3-214.3	97.1-139.9	50.2-69.4	2-22
21-30	39-64	0	10	216.8-235.8	108.4-157.5	49.2-68.8	2-27
31-40	44-69	1	9	238.8-246.8	114.4-158.8	47.6-64.8	2-18
41-50	32-65	1	9	251.5-271.8	118.6-189.4	46.2-75.3	2-25
51-59	42-73	1	8	275.8-416.5	139.9-225.9	42.9-63.7	5-32
Average	51			235.4	140.9	59.4	11

\*The data in the above table have been grouped in order to conserve space.

TABLE II

THE TOTAL CHOLESTEROL CONTENT OF THE PLASMA IN NORMAL SUBJECTS AND IN PATIENTS WITH ARTHRITIS: STATISTICAL ANALYSIS

GROUP	NUMBER OF OBSERVATIONS	ARITHMETIC MEAN MG. PER 100 C.C.	STANDARD* DEVIATION	PROBABLE† ERROR OF MEAN	$\frac{d}{\sigma_D}$
Normals	33	194.7	29.3	3.42	0.0
Rheumatoid arthritis	33	175.2	39.5	4.60	2.27
Osteoarthritis	59	235.4	45.1	3.69	5.20

$$*Standard deviation \sigma = \sqrt{\frac{\sum (d^2)}{N}}$$

Where  $\sum (d^2)$  represents the summation of the squares of the individual deviations from the mean, and N the number of determinations.

$$\dagger Probable error of mean = 0.6745 \frac{\sigma}{\sqrt{N}}$$

Where  $\sigma$  represents the standard deviation, and N the number of determinations.

$\dagger \frac{d}{\sigma_D}$  represents the difference between two means divided by the standard error of the difference.

The standard error of the difference  $\sigma_D$  is calculated from the formula

$$\sigma_D = \sqrt{\frac{\sigma_1^2}{N_1} + \frac{\sigma_2^2}{N_2}}$$

Where  $\sigma_1$  and  $\sigma_2$  represent the standard deviations of the two groups and  $N_1$  and  $N_2$  represent the number of determinations in the two groups.

The blood specimens were obtained by venipuncture, usually after breakfast. Free and ester cholesterol were determined by the Bloor-Knudson<sup>5</sup> method. Occasionally, the total plasma cholesterol was determined by the Sackett<sup>6</sup> modification of the Bloor method. Colorimetric determinations were carried out according to the temperature control procedure adopted in this laboratory.<sup>7</sup> The sedimentation rate was determined by the method of Westergren.<sup>8</sup> In many cases, the sedimentation rate and the plasma cholesterol were determined simultaneously; on the other hand, no correlation between cholesterol and sedimen-

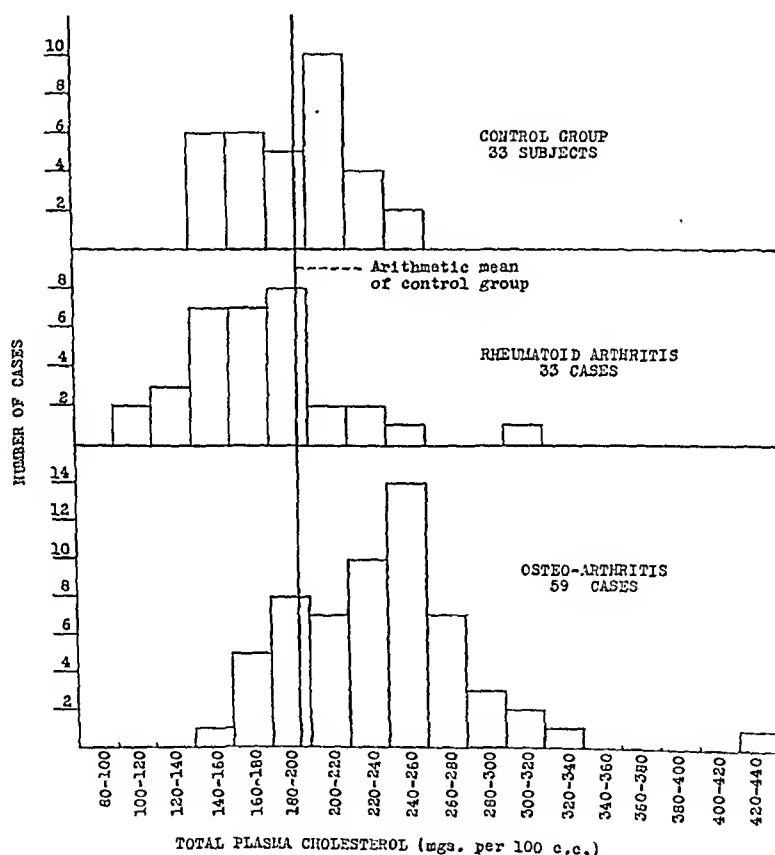


Fig. 1.—Frequency curve. The total cholesterol content of the plasma in normal subjects and in patients with rheumatoid arthritis and osteoarthritis.

tion rate was attempted if more than thirty days intervened between the individual determinations.

In the present investigation, the plasma cholesterol in subjects with arthritis has been compared with cholesterol studies in thirty-three normal subjects, all determinations being carried out under identical conditions in the same laboratory. As has been asserted in previous communications from this laboratory,<sup>4, 9</sup> we believe the cholesterol range in normal subjects to lie between 160 and 230 mg. per 100 c.c. of plasma, values from 230 to 250 mg. are only suggestive of hypercholesterolemia, figures above 250 mg. are considered to be definitely elevated and those below 160 mg. to be distinctly below the normal level.

The formulas used in the statistical analysis of our data are indicated in Table II.

### RESULTS

Table I shows the results obtained in 33 cases of rheumatoid arthritis and in 59 cases of osteoarthritis.

In the rheumatoid group the average age was thirty-nine years; 63 per cent of the patients were females. The mean total cholesterol was  $175.2 \pm 39.5$  mg. per 100 c.c. of plasma. When these figures were compared with the control group (see Table II and Fig. 1), the results indicated that 49 per cent of the rheumatoid cases gave normal values, 39 per cent showed hypocholesterolemia,

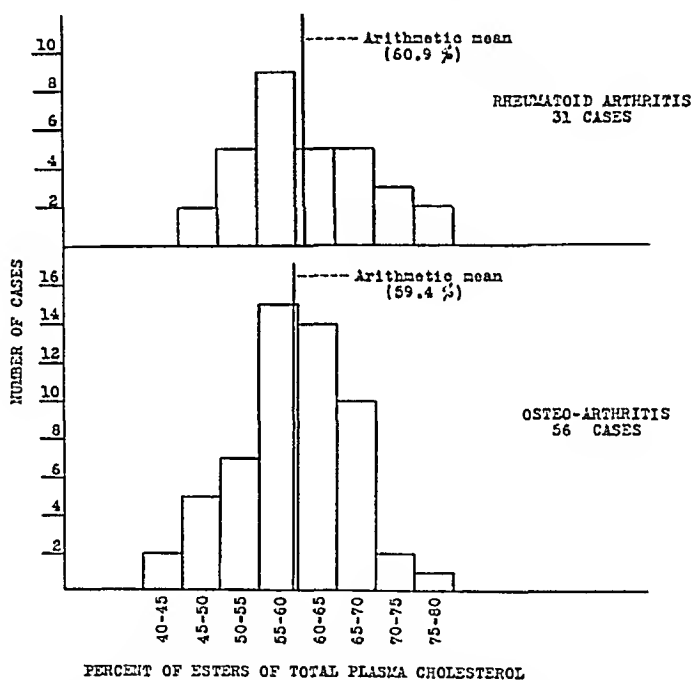


Fig. 2.—Frequency curve. The per cent of esters of the total plasma cholesterol in patients with rheumatoid arthritis and osteoarthritis.

and 12 per cent hypercholesterolemia. The esterified cholesterol showed a normal relation to the total cholesterol varying from 46.6 per cent to 76.8 per cent with a mean of 60.9 per cent. No absolute correlation was observed between the total plasma cholesterol and the sedimentation rate.

In the osteoarthritis group the average age was fifty-one years; 90 per cent of the patients were females. The mean total cholesterol was  $235.4 \pm 45$  mg. per 100 c.c. of plasma. Sixty-two per cent of the cases had hypercholesterolemia, 35 per cent gave normal values, and only 3 per cent showed hypocholesterolemia. The cholesterol esters in this group also showed a normal relation to the total cholesterol, varying from 42.9 per cent to 75.3 per cent with a mean of 59.4 per cent. Here again, no definite correlation was observed between the sedimentation rate and the total blood cholesterol.

Table II presents a statistical analysis obtained in the three groups of subjects. This analysis is included in the present study to show that the results derived from this investigation are at least suggestive. The arithmetic means and the standard deviations indicate the trend, the plasma cholesterol tends to be low in rheumatoid arthritis and elevated in osteoarthritis. That these results, especially those obtained in the osteoarthritis group, are really significant is evident from the final calculations; the difference between the means of the normal group and the rheumatoid group divided by the standard error of the difference of these means gives a value of 2.27. This variation from normal is suggestive but not definite. The same calculations applied to the normal and osteoarthritis group show a figure of 5.20, indicating a significant variation from normal.

Figs. 1 and 2 present frequency curves of the data given above. They are self-explanatory and require no further elucidation.

#### DISCUSSION

The etiology of rheumatoid arthritis is unknown; the more recent clinical and laboratory data point to its infectious origin. The type of infection and its mode of action are still in dispute; the pathologic findings, however, would suggest either a hematogenous infection or an allergic response as the prime factor in the causation of this type of arthritis. The observation recorded in this paper that patients with rheumatoid arthritis tend to show a low blood cholesterol supports the infectious theory, since other acute infections are accompanied by hypocholesterolemia (Denis,<sup>10</sup> Kipp,<sup>11</sup> Stepp,<sup>12</sup> Rosen and Krasnow,<sup>13</sup> Eichelberger and McCluskey,<sup>14</sup> Henning<sup>15</sup>).

The etiology of osteoarthritis, likewise, is not definitely known. The consensus of opinion is that the pathologic manifestations suggest the degenerative origin of this disease. The tendency to an elevated blood cholesterol in this condition supports this view, since other degenerative diseases are usually accompanied by hypercholesterolemia (Bruger and Poindexter<sup>4</sup>).

The relation of free to ester cholesterol in the plasma of arthritides is not disturbed. The mean percentage of esters of the total plasma cholesterol in the two groups of arthritis (60.9 per cent in rheumatoid arthritis and 59.4 per cent in osteoarthritis) is almost identical with that given by Bloor and Knudson<sup>16</sup> (58 per cent) for normal subjects.

The sedimentation rate in rheumatoid arthritis is usually increased, the rapidity of sedimentation being roughly proportional to the activity of the disease. We hoped to observe an inverse ratio between the plasma cholesterol and the sedimentation rate in these cases, since both determinations appeared, at first hand, to be an index of the severity of the disease. As stated above, no such correlation was observed, although one might venture to say that extremely low plasma cholesterol (as we have encountered in the acute phases of rheumatoid arthritis and in acute rheumatic fever) are usually accompanied by an increased rate of blood cell sedimentation. The converse, that is, a high cholesterol and a normal or low sedimentation rate was not obvious from our figures in the rheumatoid group, though suggestive in the osteoarthritis group.

In the first analysis, two factors may account for the elevated blood cholesterol in osteoarthritis. First, these patients are usually obese, and second, they generally fall into an older age group. In a previous communication from this laboratory,<sup>4</sup> we have shown that uncomplicated obesity (that is, obesity in which evidence of any complicating degenerative disease is lacking) is associated with a normal plasma cholesterol. Again, there are few pertinent studies in the literature which point to an increased blood cholesterol in the aged. Parhon and Parhon<sup>17</sup> are of the opinion that in human subjects there is only a slight increase in the blood cholesterol with advancing years. In very old people (over seventy years of age) they observed a definite hypercholesterolemia. In a group of fourteen normal subjects reported by Gorham and Myers,<sup>3</sup> three were fifty-one, sixty-five, and fifty-five years old, respectively, and the blood cholesterol in these three patients ranged from 130 to 170 mg. per 100 c.c. We have frequently encountered normal plasma cholesterols in old subjects and are of the opinion that the factor of age alone in no way influences the level of the blood cholesterol in human subjects. When arteriosclerosis supervenes, however, the cholesterol content of the plasma may increase, but even the advent of this condition is not invariably accompanied by an elevation of the blood cholesterol (Gorham and Myers,<sup>3</sup> Peters and Van Slyke<sup>18</sup>). It seems correct to conclude, therefore, that the two factors of obesity and of age may be eliminated as possible causes for the increased blood cholesterol observed in patients with osteoarthritis.

#### SUMMARY AND CONCLUSIONS

The total cholesterol content of the plasma tends to be decreased in rheumatoid arthritis and elevated in osteoarthritis. These findings lend added proof to the theory that rheumatoid arthritis is an infectious disease and osteoarthritis a degenerative one. The relation of free to ester cholesterol in the plasma is not disturbed in patients with arthritis; the cholesterol partition of the plasma in all cases of arthritis approaches closely to that observed in normal subjects. There is no absolute correlation between the sedimentation rate and the total blood cholesterol in patients with arthritis; at times, very low plasma cholesterols are associated with elevated sedimentation rates.

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## OBSERVATIONS ON THE INDICAN TEST ON THE BLOOD AND URINE IN RENAL INSUFFICIENCY\*

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S. H. POLAYES, PH.B., M.D., AND ELIZABETH ANN ECKERT,  
BROOKLYN, N. Y.

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IN 1911, Obermeyer and Popper<sup>1</sup> first called attention to the fact that indican is increased in the blood of patients with uremia. This was soon confirmed by the observations of Tschertkoff<sup>2</sup> and others, whose investigations showed that a quantitative determination of indican in the blood could be used as a valuable aid in detecting renal insufficiency. Thus Baar,<sup>3</sup> in a monograph on indicanemia, asserts that indican retention is the most accurate of all evidences of disturbed renal function. He based this assertion on the observation that in an individual with normal kidneys the blood indican remains constant regardless of the amount of indican absorbed from the intestines,<sup>4</sup> whereas if the kidneys are impaired, a marked indicanemia occurs, even in the absence of intestinal putrefaction. Rosenberg<sup>5</sup> reports indican values as high as 7 mg. per 100 c.c. of blood in patients with renal insufficiency. (The normal figures are 0.026 to 0.085 mg. per 100 c.c. of blood.) The frequent association of indicanemia with renal insufficiency has also been pointed out by Schilling and Holzer,<sup>6</sup> Becher,<sup>7</sup> Eufinger and Bader,<sup>8</sup> Beeher, Litzner, and Doenecke,<sup>9</sup> Muto,<sup>10</sup> Harrison and Bromfield<sup>11</sup> and others.

The observations of Krociewicz<sup>12</sup> and others<sup>13, 14</sup> point to the possibility that the quantitative estimation of the blood indican may be of aid in the diagnosis of renal disease even before retention of nonprotein nitrogen begins. It has also been claimed that, since indican accumulates in the blood only when there is destruction of renal parenchyma, the test may be used as an

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\*From the Department of Pathology, Jewish Hospital of Brooklyn, Dr. Max Lederer, Director.

Received for publication, July 25, 1934.

aid in differentiating true renal insufficiency from conditions with nonprotein nitrogen retention which is not due to renal disease primarily. Thus, the observations of Monias and Shapiro<sup>15</sup> have led them to conclude that the accumulation of indican in the blood is a sensitive and specific test of absolute renal insufficiency, reliable in both diagnosis and prognosis. Salvini<sup>16</sup> has recently arrived at a similar conclusion in studying a series of cases showing an increased indicanemia following operation for tumors of the uterus and ovaries.

Should the above observations be confirmed, the clinician would be provided with a much desired test for the diagnosis of early renal disease. The importance of such a test is obvious, since the various tests for renal insufficiency at present employed are of value in detecting only advanced renal disease. It is only occasionally that they serve as an aid in diagnosis of early renal impairment.

Following the report on indicanemia in renal disease by Monias and Shapiro in 1930, we made quantitative determinations on the blood indican in a series of cases of renal insufficiency. It soon became apparent that not all individuals with renal dysfunction and pronounced nonprotein nitrogen retention showed an increase in the blood indican. This observation showed the necessity for further investigation of this subject. Routine quantitative indican determinations were, therefore, performed on all bloods received in our laboratory for chemical examination and, at the same time, quantitative indican determinations were made on the urine of each of these patients.\* A record was also made of the results of other tests of renal function which were performed on these patients (urinalyses, phenolsulphonephthalein excretion and the diazo reaction on the blood) and of any significant clinical signs of renal disturbances whenever such signs were present. Three hundred cases were analyzed in all, of which 241 showed no clinical or laboratory evidence of renal dysfunction. Of the remaining 59 cases some showed varying degrees of renal insufficiency, clinically, and all showed an increase of one or more of the nonprotein nitrogen constituents of the blood (urea nitro-

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\*Jolles<sup>17</sup> modification of the Obermeyer and Popper method was employed in this study, using the artificial standard of Monias and Shapiro. The technic is as follows: Five cubic centimeters of serum are placed in an Erlenmeyer flask and to this is added an equal quantity of distilled water. An equal quantity (10 c.c.) of trichloroacetic acid (20 per cent) is then added and the flask is rotated for about two minutes and then allowed to stand for about five minutes. The contents are then filtered through a funnel into a 100 c.c. graduated cylinder. Ten drops of a 5 per cent alcoholic solution of thymol are added to the clear filtrate to which concentrated fuming, chemically pure, hydrochloric acid containing 0.5 per cent ferric chloride is added in a quantity equal to that of the amount of filtrate obtained. The mixture is well shaken and allowed to stand for two hours. Then 5 c.c. of chemically pure chloroform are added and the mixture is shaken for two or three minutes and allowed to stand. The chloroform which settles at the bottom with the dissolved indigo is now pipetted off and the intensity of its coloration is compared with the artificial standard (6 capillary drops of a 1 per cent aqueous solution of gentian violet, 3 drops of a 1 per cent aqueous solution of eosin, 10 c.c. of 94 per cent alcohol and 30 c.c. of distilled water) which corresponds in tint and intensity to the original standard of 5 mg. of indican in 100 c.c. of chloroform. When necessary, dilutions of the standard are made with water and the dilutions taken into account in calculating the results.

The calculations are made from the formula:

$$\frac{\text{Reading of the standard} \times 0.5}{\text{Reading of the unknown}} \times 10 = \text{mg. of Indican per 100 c.c. of blood.}$$

The method employed for the quantitative determination of the indican in the urine is the same as for blood.

Note: Recently Schlierbach<sup>18</sup> recommended the use of Palfrich's step photometer for quantitative indican determinations which may be controlled by Lange's electric light colorimeter. The advantage claimed for this test is that it obviates the necessity for using photosensitive comparative solutions.



gen of 30 mg. or more and creatinine of 3 mg. or more per 100 c.e. of blood was considered evidence of retention).

The former group (cases with normal renal function and no retention of nonprotein nitrogen) can be dismissed with the statement that in no instance was a definite increase of indican found in the blood.

The latter group (cases with definite retention of nonprotein nitrogen in the blood and with or without clinical evidence of renal disease) was divided according to the blood indican content, into two subgroups, one consisting of 21 cases with normal and the other, of 38 cases, with increased blood indican values. The results of this analysis can be studied best from Tables I and II.

#### DISCUSSION OF TABLE I

A study of Table I reveals the most significant findings. Four, or about 20 per cent of the 21 cases in which the indican values of the blood were normal (Cases 34, 68, 72, and 94), showed marked renal pathology at autopsy. In Case 34 the amount of renal parenchyma which remained normal was very meager, and in Cases 72 and 94 an arteriosclerotic process had destroyed the major portion of renal parenchyma. In Case 72 there were diffuse vascular lesions in the kidneys and large portions of renal parenchyma had been destroyed by infarcts. Thus in four proved cases of advanced renal pathology the blood indican values were normal. It is significant, however, that in none of these cases was the nonprotein nitrogen retention excessive. The urea nitrogen values ranged only from 31.2 mg. to 52.13 mg. and the creatinine only from 2.4 mg. to 2.9 mg. per 100 c.e. of blood. It is also noteworthy that the Diazo test on the blood was negative in all of these 4 cases as well as in all except one (Case 97) of the remaining 17 cases of this group. This incidentally confirms a previous publication by one of us (P) on the value of the Diazo test in nephritis,<sup>19</sup> where it was pointed out that the test is not always positive even in cases of advanced renal pathology.

In six other cases (Cases 30, 49, 57, 75, 83, and 97) of this group, the diagnosis of renal disease was made from clinical signs, irrespective of the definite nonprotein nitrogen retention which was present in all of them, yet in no instance was the blood indican increased. Up to the time of writing two of these patients had died, one from diabetes and chronic nephritis and the other from cardiac decompensation and uremia. The rest were discharged either as cured or improved.

The remaining eleven cases (Cases 56, 58, 59, 62, 64, 77, 84, 87, 95, 101, and 105) of this group consisted of miscellaneous conditions. Although in none of those instances was there sufficient clinical evidence for a definite diagnosis of renal insufficiency, the persistent albuminuria and the moderate nonprotein nitrogen retention which was found in all of these cases suggested renal dysfunction. (Necropsy was performed in only one of these cases and revealed no evidence of renal pathology.)

From an analysis of the above data it is quite apparent that at least in some instances the absence of an increased indicanemia does not exclude the existence of early or even advanced renal disease. This observation, of course,

TABLE I  
CASES WITH NORMAL INDIAN VALUES IN THE BLOOD

OTHER TESTS OF BLOOD FUNCTION				P. S. P.	URINE		DIAGNOSIS ON DISCHARGE	POSTMORTEM FINDINGS AND OTHER COMMENTS
W.B.C.	HGB.	SEDIMENT	ESR.		GLUC.	ALBUMIN		
44	44	12	Traces (2 hr.)	Neg.	Neg.	3+	Pylonephritis	Recovered after drainage. (No record of subsequent chemical analysis)
44	44	12		Neg.	Neg.	3+	Coronary thrombosis	Autopsy: One kidney almost replaced by fibrosis, with atrophy of its ureter. Other kidney showed marked arteriosclerosis
44	44	Traces	66%	Neg.	Traces	3+	Chr. nephritis, Diabetes	Died
44	44	4.7	8%	Neg.	Traces	3+	Coronary closure	Improved
44	44	3.6		Neg.	Traces	3+	Nephrosclerosis	Improved
44	44	3.9		Neg.	Traces	3+	Acute appendicitis	Recovered
44	44	3.2		Neg.	Traces	3+	Gen. arteriosclerosis	Unimproved
44	44	3.2		Neg.	Traces	3+	Ca. of bladder, Chr. bronchitis	Died
44	44	9.5		Neg.	Traces	3+	Gen. art. sclerosis	Died
44	44	2.9	39%	Neg.	Traces	3+	Cirrhosis of liver	Autopsy: Periaortitis nodosa with infarcts in kidneys
44	44	7.2		Neg.	Traces	3+	Periaortitis nodosa, Hypertension	Autopsy: Coronary sclerosis, nephrosclerosis
44	44	1.0		Neg.	Traces	3+	Nephrosclerosis, Cardiac decompensation	Improved
44	44	6.4		Neg.	Traces	3+	Glomerulonephritis	Improved
44	44	4.2		Neg.	Traces	3+	Hypertrophy of prostate	Improved
44	44	1.8		Neg.	Traces	3+	Chronic nephritis	Improved
44	44	3.4		Neg.	Traces	3+	Pneumonia	Cured
44	44	3.4		Neg.	Traces	3+	General arteriosclerosis	Died
44	44	5.2		Neg.	Traces	3+	General arteriosclerosis, Nephrosclerosis	Autopsy: Nephrosclerosis; general arteriosclerosis
44	44	2.1		Neg. Pos.	Traces	3+	Subacute bacterial endocarditis	Died
44	44	5.2		Neg.	Traces	3+	Cardiac decompensation, Uremia	Died
44	44	3.9		Neg. Serum jaundiced	Traces	3+	Hypertension arteriosclerosis, Cholelithiasis and liver necrosis	Died
44	44	0.02		Neg.	Traces	3+		Autopsy: Kidneys normal. The figures given here are from tests performed three days autemortem. About a month previously the findings were normal

TABLE II  
CASES WITH INCREASED INDICAN VALUES IN THE BLOOD

TOTAL NUMBER OF CASES	MG. PER 100 C.C.				OTHER TESTS OF RENAL FUNCTION			DIAGNOSIS WITH NUMBER OF CASES OF EACH	RESULTS
	BLOOD		URINE		BLOOD	URINE			
	UREA N.	CREAT.	INDICAN	P. S. P.		ALBUMIN			
38	Average 57.1	Average 5.9	Average 2.1	Average 4.3	Positive in all except Case 50	Traces (t e s t) was perform- ed in only 12% of these cases)	Average 3+	Chronic glomerulonephritis	25
	Range 19.6-250.0	Range 1.7-25.0	Range 0.1-7.4	Range traces-- 12.5				Nephrosclerosis Acute glomerulonephritis Urinary obstruction { Hypertrophied prostate { Vesical calculi Polycystic kidneys Hypertension Subacute bacterial endo- carditis	5 2 2 1 1 3 1
									Died 24 cases Improved 12 cases Unimproved 2 cases

militates against the reliance which has been placed on this test as a means of diagnosis of incipient or even of advanced renal pathology. Thus, in the four cases cited above (Cases 34, 68, 72, and 94), the moderately increased nonprotein nitrogen values of the blood, together with the other tests of renal function, proved to be more informative than did the blood indican determinations.

#### DISCUSSION OF TABLE II

It was stated above that early in the course of this investigation it had been observed that an increase in the blood indican was not found in any of the cases with normal nonprotein nitrogen values. Neither was an increased blood indican found in any of the cases in Table I, where, as was just pointed out, an increased indicanemia was expected, since all of these cases showed evidence of renal insufficiency varying in degree from mild to fatal forms. It was, therefore, deemed advisable to tabulate separately all the cases which did show an increase in the blood indican and thereby permit of a more accurate comparative study of the two groups. In Table II, therefore, are recorded only those cases which showed an increase in the blood indican. A study of this table reveals that with one minor exception, all of these cases of this group showed marked nonprotein nitrogen retention in the blood, positive blood diazo reactions, poor phenolsulphonaphthalein excretion, marked and persistent albuminuria, and clinical evidence of advanced renal disease. In other words, an increase in blood indican occurred only in cases with definite nonprotein nitrogen retention and other clinicopathologic evidences of renal pathology. Of the 38 patients examined in this group, 22 or about 60 per cent were cases of chronic glomerulonephritis. The rest consisted of the following: 5 cases of nephrosclerosis; 3 cases of so-called essential hypertension terminating in uremia; 2 cases of acute glomerulonephritis, one of which was superimposed on a chronic glomerulonephritis; 3 cases of urinary obstruction, two due to hypertrophy of the prostate and one due to vesicle calculi; 2 cases of polycystic kidneys and one case of subacute bacterial endocarditis. Twenty-four, or nearly 63 per cent, of these patients died after a course of a number of weeks or days at the hospital. Postmortem examinations were performed in two of the cases, one a case of nephrosclerosis and the other a case of chronic glomerulonephritis. In both instances the findings confirmed the clinical impression, marked destruction of renal parenchyma having been found in both. It is of interest to note that in a number of instances, while the retained nonprotein nitrogen of the blood remained constant or was diminishing, the clinical condition of the patient was rapidly progressing to a fatal termination. In these instances it was observed that while the indican of the blood increased the indican of the urine diminished. Findings in Case 32 serve to illustrate this observation (Table III).

In cases in which the urine indican fails to decline synchronously with a rise in the blood indican, a fatal prognosis cannot be made with nearly as much certainty. A persistent increase in the urine indican, on the contrary, signifies renal ability to excrete this toxic substance and, given sufficient time, the excess accumulation of the blood indican may be eventually eliminated.

The group of cases with urinary obstruction deserves special comment. In all of these cases the blood findings, including the indican values, returned

TABLE III

MG. PER 100 C.C.			
BLOOD		URINE	
UREA	CREATININE	INDICAN	
125.0	10.0	0.56	9.8
81.3	10.0	5.20	Traces
52.4	10.0	7.20	Traces

to normal soon after the cause of obstruction was removed in each case. Apparently, no permanent renal damage had been produced by the obstruction. The increase of the indican and nitrogenous waste products in the blood was, therefore, only transitory. This observation again illustrates the fallacy of attaching prognostic or diagnostic significance to the results of single blood chemical determinations.

Another observation worthy of comment is that the indican determination may be made on blood secured postmortem and also on blood which had been preserved in a refrigerator for an indefinite period of time. Determinations made on specimens of blood so obtained or preserved yield values which, for practical purposes, are the same as those derived from the examination of blood obtained during life. Control experiments similar to those recently described by one of us (P) in a report on postmortem blood chemistry in renal disease<sup>20</sup> showed that the results of such examinations are reliable and may be used without hesitation when, for various reasons, it may be impossible to obtain fresh blood for analysis.

#### SUMMARY AND CONCLUSIONS

Quantitative indican determinations were made on the blood and urine of 300 hospital patients, all of whom were observed for evidence of renal disease. Blood urea nitrogen and creatinine determinations and other tests of renal function were made in each case. Based on the results of these examinations the cases were divided into two groups. One group consisted of 241 cases none of which showed any evidence of renal disease. The other group consisted of 59 cases all of which showed definite nonprotein nitrogen retention in the blood and most of which showed also clinical evidence of renal pathology. These 59 cases were subdivided into two subgroups, one consisting of cases with normal, the other with increased blood indican values. The conclusions drawn from this investigation were as follows:

1. Increases in the blood indican values were always accompanied by increases in the blood nonprotein nitrogen content.
2. The blood indican values remained normal in a considerable number of instances in which the nonprotein nitrogen of the blood was definitely increased and in which clinical, laboratory and finally, postmortem examination revealed evidence of severe renal disease.
3. A persistent increase in the blood indican values occurring concomitantly with a persistently diminishing indican content in the urine was found

to be significant of a rapidly fatal prognosis, and in that respect, more reliable than determinations of the nonprotein nitrogen content of the blood. About two-thirds of the patients with a persistent increase in the blood indican died during the course of the investigation.

4. Increases in the blood indican were found to be only temporary in several instances of urinary obstruction. Removal of the cause of obstruction was immediately followed by a return of the blood indican values to normal.

5. Indican determinations may be made on postmortem or preserved blood.

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## BACTERIOSTATIC AND BACTERICIDAL STUDIES OF VARIOUS DYES AND ALLIED COMPOUNDS\*

S. A. PETROFF, PH.D., AND WILLIAM S. GUMP, PH.D., TRUDEAU, N. Y.

THE indiscriminate use of various compounds in the treatment of tuberculous empyema, and especially the form complicated with pus-forming microorganisms, prompted these studies. Numerous attempts both clinical and experimental have been made to sterilize the pleural cavity, but up to the present not a single known compound has proved of great value in such therapy. It is true there are a number of effective organic mercurial compounds which exert bactericidal properties in vitro, but as soon as they come in contact with body fluids this property becomes nullified, and they are of little value. The demand for an effective drug for such therapy has so increased in recent years, that it could not very well be ignored, but at the outset we fully realized the difficulties intimately connected with it.

A general survey of the subject revealed that a number of compounds with established bacteriostatic and bactericidal properties completely failed to bring about desired results when used for therapeutic purposes. This fairly well signifies that tests as performed today do not give us the true germicidal index of the drug. Usually ordinary beef infusion broth and agar media have been used, and at the best, they are unsuited for a study of this nature. The medium for vitro experiment should be one which will approach the conditions under which the compound will be used. To conform with this we used throughout our studies human serum agar media.

The bacteriostatic property of some 130 dyes and allied compounds were studied, selecting the effective ones for the bactericidal tests. Of the organisms used 10 were gram-positive (staphylococci, pneumococci, and streptococci), and 7 were gram-negative (typhosus, paratyphosus, dysentery, coli and vibrio comma). Several groups contained more than one strain. The object was to find out, for example, if *Pneumococcus* I, II, and III were equal in their susceptibility to a given drug.

### A. ORGANIC DYES

#### I. Nitroso Dyes:

##### 1. Naphthol green B

#### II. Nitro Dyes:

##### 2. Martius yellow

##### 3. Naphthol yellow S

\*From the Research and Clinical Laboratory.  
Received for publication, August 24, 1934.

III. *Azo Dyes:*

## (a) Monoazo.

4. Azo bordeaux
5. Bordeaux red
6. Chrysoidine R
7. Chrysoidine Y
8. Naphthylamine brown
9. Naphthylamine yellow
10. Naphthylamine red
11. Trypan blue
12. Trypan red
13. Vital red
14. Azo blue
15. Chrysamine

## (b) Diazo.

IV. *Stilbene Dyes:*

16. Chrysophenine

V. *Triphenylmethane Dyes:*

## (a) Acid.

17. Alkali blue
18. Aniline blue
19. Brilliant acid blue
20. Neutral violet

## (b) Basic.

21. Brilliant green
22. Gcutian violet
23. Methyl violet 6B
24. Iodine green
25. Methyl green
26. Basic fuchsin
27. Para rosaniline base
28. Para rosaniline sulphate
29. Para rosaniline acetate
30. Rosaniline hydrochloride
- \*31. Para rosaniline salicylate
- \*32. Isoamyl-para rosaniline iodide
33. Ethyl violet
- \*34. Ethyl violet bromide
- \*35. Ethyl violet iodide
- \*36. n-Propyl violet (homologue of ethyl violet)

VI. *Xanthene Dyes:*

## (a) Pyronines.

37. Acridine red

## (b) Fluoresceins.

38. Phloxine

## (c) Rhodamines.

39. Rhodamine B
40. Rhodamine G
41. Rhodamine 6G

VII. *Acridine Dyes:*

42. Acridine yellow

- \*43. Acridine yellow salicylate

44. Acridine orange

45. Proflavine

46. Neutral acriflavine

47. Acriviolet

VIII. *Quinoline Dyes:*

48. Quinoline yellow

49. Ethyl red

50. Ethyl cyanine T

51. Orthochrome T bromide

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\*Prepared in our laboratory.



- 52. Pinacyanole
- 53. Pinaflavol
- 54. Quinaldine red

#### IX. Azine Dyes:

- (a) Chinoxalines.
  - 55. Pinacryptol green
  - 56. Methylene heliotrope
  - 57. Methylene violet 3 RA extra
  - 58. Safranine purple M
  - 59. Safranine Y
  - 60. Indoine blue B B
  - 61. Iris violet
  - 62. Induline scarlet
- (c) Nigrosines.
  - 63. Nigrosine

#### X. Oxazine Dyes:

- 64. Nile blue
- 65. Gallocyanine
- 66. Alizarine green G
- 67. Cresyl violet

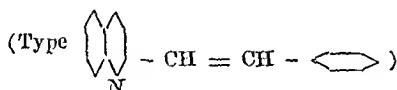
#### XI. Thiazine Dyes:

- 68. Methylene blue
  - 69. New methylene blue N
  - 70. Thionine
  - \*71. Ethylene blue
  - \*72. n-Propylene blue
  - \*73. n-Butylene blue
- } homologues of methylene blue

#### XII. Anthraquinone Dyes:

- 74. Alizarine blue S
- 75. Alizarine red S

#### B. STYRYLQUINOLINE COMPOUNDS

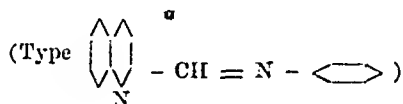


#### XIII.

- (a) Hydrochlorides.
  - \*76. Benzylidene quinaldine
  - \*77. Benzylidene-4-methyl quinaldine
  - \*78. Benzylidene-6-methyl quinaldine
  - \*79. Cinnamylidene quinaldine
  - \*80. o-Aminocinnamylidene quinaldine
  - \*81. Benzylidene-6-diethylamino quinaldine
  - \*82. Piperonylidene-6-diethylamino quinaldine
  - \*83. o-Hydroxybenzylidene quinaldine
  - \*84. p-Hydroxybenzylidene quinaldine
  - \*85. 2(β-Furfuryl-vinyl) quinoline
- (b) Methochlorides.
  - \*86. Benzylidene-6-methyl quinaldine
  - \*87. Cinnamylidene quinaldine
  - \*88. Cinnamylidene-6-methyl quinaldine
  - \*89. o-Nitrocinnamylidene quinaldine
  - \*90. o-Chlorobenzylidene quinaldine
  - \*91. o-Chlorobenzylidene-6-methyl quinaldine
  - \*92. p-Dimethylaminobenzylidene-6-methyl quinaldine methiodide

\*Prepared in our laboratory.

## C. ANIL QUINOLINE COMPOUNDS



XIV.

- \*93. 2(p-Dimethylamino anil)- $\beta$ -naphthoquinaldine methochloride
- \*94. 2(p-Dimethylamino anil)- $\beta$ -naphthoquinaldine methonitrate
- \*95. 2(p-Dimethylamino anil)- $\beta$ -naphthoquinaldine methacetate
- \*96. 2(p-Dimethylamino anil)- $\beta$ -naphthoquinaldine methosulphate
- \*97. 2(p-Diethylamino anil)- $\beta$ -naphthoquinaldine methacetate
- \*98. 2(p-Dimethylamino-o-methyl anil)- $\beta$ -naphthoquinaldine metho-  
sulphate
- \*99. 2(p-Dimethylamino-o-methyl anil)- $\beta$ -naphthoquinaldine meth-  
acetate
- \*100. 2(p-Dimethylamino-o-chloro anil)- $\beta$ -naphthoquinaldine meth-  
acetate
- \*101. Condensation product of  $\beta$ -naphthoquinaldine methosulphate  
and 6-Nitroso-N-ethyl-tetrahydroquinaldine
- \*102. Condensation product of  $\beta$ -naphthoquinaldine methiodide and  
6-Nitroso-N-ethyl-tetrahydroquinaldine
- \*103. Condensation product of  $\beta$ -naphthoquinaldine methacetate  
and 6-Nitroso-N-ethyl-tetrahydroquinaldine
- \*104. Condensation product of  $\beta$ -naphthoquinaldine methacetate  
and 6-Nitroso tetrahydroquinaldine
- \*105. 2(p-Monoethylamino-m-methyl anil)- $\beta$ -naphthoquinaldine  
methacetate
- \*106. 2(p-Dimethylamino anil)-4-ethoxy- $\alpha$ -naphthoquinaldine methi-  
odide
- \*107. 2(p-Dimethylamino anil)-4-ethoxy- $\alpha$ -naphthoquinaldine metho-  
sulphate

## D. QUINOLINE DERIVATIVES

XV.

- 108. Quinosol
- \*109. 8-Hydroxyquinaldine hydrochloride
- \*110. 2-Ethyl-3-methyl-4-anilino-quinoline hydrochloride
- \*111. 2-Propyl-3-ethyl-4-anilino-quinoline hydrochloride
- \*112. 2(Phenylisoaminomethyl)-3-chloro-4-anilino-quinoline hydro-  
chloride
- \*113. 2(Chloromethyl)-3-ethyl-4-anilino-quinoline hydrochloride
- \*114. Quinaldyl-tetraethyldiaminodiphenylmethane hydrochloride
- \*115. Quinaldyl-tetraethyldiaminodiphenyl carbinol hydrochloride
- \*116. 2-Trichloroethylidene quinaldine hydrochloride
- \*117. Quinoline-2-acrylic acid
- \*119. 2-Crotonylidene quinaldine hydrochloride
- \*120. Methiodide of 2- $\beta$ -naphthoquinalddehyde pheuyllhydrozone
- \*121. 2(Chloromethyl)-3-chloro-4-(p-toluidino)-6-methyl quinoline  
hydrochloride
- \*122. 5, 7-Dinitro-8-hydroxyquinoline
- \*123. 5, 7-Diamino-8-hydroxyquinoline hydrochloride

\*Prepared in our laboratory.

## E. ALKALOIDS AND DERIVATIVES

XVI.

- 124. Harmine
- 125. Isoquinine
- 126. Aminohydroquinine
- 127. Ethylapoquinine
- \*128. 5-Amino-8-phenylazo-dihydroquinine
- \*129. 5-Amino-8-(2-chlorophenyl)-azo-dihydroquinine
- \*130. 5-Amino-8-(3-chlorophenyl)-azo-dihydroquinine

*Methods Employed.*—The bacteriostatic tests were carried on Petri plate media. Churchman's<sup>1</sup> method of divided plates, that is, one-half containing medium with dye and the second half acting as control, proved satisfactory. The original method as introduced by Churchman was slightly modified and proved satisfactory for preparation of the plates. Small strips of stiff cardboard 0.5 cm. wide were cut slightly longer than the inside diameter of the dish. When placed crosswise in the bottom part of the dish, there will be two compartments. If the strip is of the proper length, it will bulge slightly in the center with spring effect to keep itself in place. The cover is put on and the dish sterilized at 150° C. for two hours.

To melted pneumococcus agar of pH 7.6, which has been cooled to 45° C., pleural fluid to 10 per cent was added and kept at this temperature until used.

Into one compartment in the Petri plate prepared as described about 10 c.c. of the melted medium is poured, the cardboard division preventing the medium running through the entire dish. After solidification a straight diametrical cut is made in the medium with a sterile platinum spade, and with a sterile forceps the cardboard is lifted, carrying away some of the adhered medium. This manipulation results in a perfect half plate which is the control.

To a definite amount of melted serum agar, the dye is added to make a dilution ranging from 25,000 to 200,000 for gram-positive organisms, and 5,000 to 100,000 for the gram-negative organisms. First the dyes were dissolved either in water or 95 per cent alcohol and then added to the melted serum agar to the desired dilution, and 10 c.c. of this poured into the second compartment following the same method. The plates will be in perfect halves, one containing the serum agar control and the other the medium with the dye to be studied.

In order to conserve media as much as possible, six or seven streaks were made on each plate. To control the proper distribution of the streak, the bottom of each plate was ruled with a special ruler which consisted of a wire (No. 14 gauge) ring with soldered cross pieces. The ruling was done with India ink. For inoculation the lines can be followed, and there is no difficulty in making six or seven streaks on a single plate. All cultures to be studied were made on pneumococcus serum broth of pH 7.6, and for uniformity, 0.1 c.c. of inoculum was used. The inoculation of the plates was made from an eighteen-hour growth with a platinum loop of 3 mm. in diameter, streaking across the two compartments, and having as a guide the lines on the bottom of the plate.

\*Prepared in our laboratory.

The plates were incubated at 37.5° C. and the first reading made at twenty-four hours. The results recorded in the table were the forty-eight-hour observations, which were made in triplicate.

In order to determine the value of a compound, in anticipation of its practical application in therapy such as we were interested in, the following gram-positive and gram-negative organisms were used. Gram-positive: *Staphylococcus pyogenes aureus*, *Pneumococcus* I, II, III, *Streptococcus hemolyticus*, *nonhemolyticus*, *scarlatina*, *viridans*, *erysipelatis* and *faecalis*. Gram-negative: *Bacillus typhosus*, *paratyphosus A* and *B*, *dysentery Shiga*, *Flexner* and *Sonne*, *coli*, and *vibrio comma*.

The results are summarized in Tables I, II, and III. To save space in the table, we have omitted 000 from the figures, therefore each number represents thousands.

Out of 130 compounds studied, 51 were effective for gram-positive, and 23 for gram-negative organisms. Studying the tables the reader will notice that our results are considerably lower than those reported by other investigators. This discrepancy in all probability is due to the difference of media. At best, on ordinary culture medium, many organisms propagate only spar-

TABLE I\*  
BACTERIOSTATIC STUDIES—GRAM-POSITIVE ORGANISMS

LAB. NO.	COMPOUND	STAPH. P. AUREUS	PNEUMO. I	PNEUMO. II	PNEUMO. III	STREP. HEMOLYTICUS	STREP. NONHEMOLYTIC.	STREP. SCARLATINA	STREP. ERYSIPELATIS	STREP. VIRIDANS	STREP. FAECALIS
21	Gentian violet	50	50	50	50	50	25	25	25	25	—
22	Ethyl violet	50	50	100	50	50	50	50	50	50	25
25	Brilliant green	100	200	200	200	100	50	50	50	50	25
26	Basic fuchsin	25	50	100	50	50	50	50	50	50	25
27	para Rosaniline acetate	50	100	100	100	50	50	50	50	50	—
28	para Rosaniline base	25	50	50	50	50	25	25	25	25	—
29	para Rosaniline sulphate	—	25	50	50	50	50	50	50	50	—
30	Rosaniline hydrochloride	—	50	50	50	50	50	50	50	25	—
31	para Rosaniline salicylate	—	50	50	50	25	25	25	25	25	—
32	Ethyl violet bromide	—	25	25	25	25	25	25	25	25	25
33	Ethyl violet iodide	—	25	50	25	25	25	25	25	25	—
34	Isoamyl-para rosaniline iodide	—	25	25	25	25	25	25	25	25	—
35	Methyl violet 6B	100	150	200	150	50	50	50	50	50	25
36	n-Propyl violet	100	150	150	150	50	50	50	50	50	25
41	Rhodamine 6G	25	25	25	25	25	25	25	25	25	—
42	Aeridine yellow	25	100	100	100	100	25	25	25	25	—
44	Proflavine	50	100	100	50	100	50	50	50	50	25
45	Neutral acriflavine	100	100	100	100	100	100	100	100	100	25
46	Aeriviolet	50	100	100	100	100	50	50	50	50	25
54	Orthochrome T bromide	25	25	25	25	25	25	25	25	25	—
55	Pinaeryptol green	—	50	50	50	50	25	25	25	—	—
56	Methylene heliotrope	50	50	50	50	50	50	50	50	50	25
57	Methylene violet 3 RA extra	100	150	150	50	25	25	25	25	25	—
59	Safranin Y	25	50	50	50	25	25	25	25	25	—
60	Indoine blue B B	100	200	200	150	50	25	25	25	25	—
61	Iris violet	—	50	50	50	25	—	—	—	—	—
64	Cresyl violet	100	150	150	150	50	50	50	50	150	25
65	Nile blue	—	25	25	25	—	—	—	—	—	—

\*The figures represent thousands.

ingly provided the medium is not enriched with some body fluids. We had no difficulty in observing a profuse growth in the control compartment of the plates with streptococcus and pneumococcus. We may offer at present the following three explanations which, singly or collectively, may have contributed to the low values of the inhibitory properties reported here. (1) The enriched medium allowing the organism uninterrupted propagation, (2) the serum having caused some direct modification of the dye molecule thus mak-

TABLE II\*  
BACTERIOSTATIC STUDIES—GRAM-POSITIVE ORGANISMS

LAB. NO.	COMPOUND	STAPH. P. AUREUS	PNEUMO. I	PNEUMO. II	PNEUMO. III	STREP. HEMOLYTICUS	STREP. NONHEMOLYTIC.	STREP. SCARLATINA	STREP. ERYSIPELATIS	STREP. VIRIDANS	STREP. FAECALIS
68	Methylene blue	100	200	200	200	100	100	100	100	100	50
69	New methylene blue N	50	100	100	100	50	50	50	50	50	25
71	Ethylene blue	100	300	300	300	150	50	100	100	25	25
72	n-Propylene blue	100	300	300	300	150	50	100	100	25	25
73	n-Butylene blue	100	200	200	200	150	50	100	100	25	25
91	p-Dimethylaminobenzylidene-6-methyl quinaldine methiodide	25	100	100	100	25	--	25	25	25	--
92	o-Chlorobenzylidene-6-methyl quinaldine		25	25	25	25	25	25	25	25	--
93	2(p-Dimethylamino anil)- $\beta$ -naphthoquinaldine methochloride		100	100	50	50	50	50	50	25	25
94	2(p-Dimethylamino anil)- $\beta$ -naphthoquinaldine methonitrate		150	200	200	200	50	150	150	25	25
95	2(p-Dimethylamino anil)- $\beta$ -naphthoquinaldine methacetate		150	200	150	150	150	150	150	50	25
96	2(p-Dimethylamino anil)- $\beta$ -naphthoquinaldine methosulphate		150	300	150	150	100	100	100	100	50
97	2(p-Diethylamino anil)- $\beta$ -naphthoquinaldine methacetate		50	150	100	100	25	100	100	25	--
98	2(p-Dimethylamino-o-methyl anil)- $\beta$ -naphthoquinaldine methosulphate		25	100	100	25	--	25	25	25	--
99	2(p-Dimethylamino-o-chloro anil)- $\beta$ -naphthoquinaldine methacetate		100	100	50	25	--	25	25	--	--
100	2(p-Dimethylamino-o-methyl anil)- $\beta$ -naphthoquinaldine methacetate		300	300	300	25	25	25	25	25	--
101	Condensation product of $\beta$ -naphthoquinaldine methosulphate and 6-Nitroso-N-ethyl-tetrahydroquinaldine		150	150	150	100	25	50	50	50	25
103	Condensation product of $\beta$ -naphthoquinaldine methacetate and 6-Nitroso-N-ethyl-tetrahydroquinaldine		100	200	150	100	25	50	50	50	25
104	Condensation product of $\beta$ -naphthoquinaldine methacetate and 6-nitroso tetrahydroquinaldine		50	50	50	50	--	50	50	50	--
105	2(p-Monoethylamino-m-methyl anil)- $\beta$ -naphthoquinaldine methacetate		50	50	50	50	25	50	50	25	--
106	2(p-Dimethylamino anil)-4-ethoxy- $\alpha$ -naphthoquinaldine methiodide		25	25	25	25	--	25	25	25	--
124	Harmine	--	100	100	100	--	--	--	--	--	--
128	5-Amino-8-phenylazo-dihydroquinine	--	25	25	25	25	--	25	25	25	--
129	5-Amino-8-(2-chlorophenyl)-azo-dihydroquinine	--	150	200	200	150	50	150	100	50	25

\*The figures represent thousands.

TABLE III\*  
BACTERIOSTATIC STUDIES—GRAM-NEGATIVE ORGANISMS

LAB. NO.	COMPOUND	B. TYPHOSUS	B. PARATYPH. A	B. PARATYPH. B	B. DYSENTERY SHIGA	B. DYSENTERY FLEXNER	B. DYSENTERY SONNE	B. COLI	VIBRIO COMMA
23	Iodine green	5	5	5	25	10	10	--	25
24	Methyl green	5	5	5	10	5	5	--	5
25	Brilliant green	50	50	50	100	50	100	50	100
26	Basic fuchsin	10	10	10	25	25	10	10	10
42	Aceridine yellow	10	10	10	25	25	25	5	10
44	Proflavine	--	--	--	10	10	10	--	5
45	Neutral acriflavine	--	--	--	50	10	25	--	--
46	Acriviolet	--	--	5	50	50	50	--	10
64	Cresyl violet	--	--	--	10	5	1	--	--
71	Ethylene blue	--	--	--	5	5	--	--	--
72	n-Propylene blue	--	--	--	10	10	--	--	5
73	n-Butylene blue	--	--	--	5	5	--	--	--
95	2(p-Dimethylamino anil)- $\beta$ -naphthoquin- dine methacetate	200	--	--	150	200	200	--	200
96	2(p-Dimethylamino anil)- $\beta$ -naphthoquin- dine methosulphate	100	--	--	150	100	150	--	100
97	2(p-Diethylamino anil)- $\beta$ -naphthoquin- dine methacetate	50	--	--	50	50	50	--	50
98	2(p-Dimethylamino-o-methyl anil)- $\beta$ -naph- thoquinaldine methosulphate	10	--	--	100	--	10	--	5
100	2(p-Dimethylamino-o-methyl anil)- $\beta$ -naph- thoquinaldine methacetate	10	--	--	25	50	25	--	25
101	Condensation product of $\beta$ -naphthoquin- dine methosulphate and 6-Nitroso-n- ethyl-tetrahydroquinaldine	5	--	--	50	50	50	--	25
103	Condensation product of $\beta$ -naphthoquin- dine methacetate and 6-Nitroso-n-ethyl- tetrahydroquinaldine	50	--	--	50	50	50	--	50
104	Condensation product of $\beta$ -naphthoquin- dine methacetate and 6-Nitroso tetra- hydroquinaldine	10	--	--	10	25	25	--	25
105	2(p-Monoethylamino-m-methyl anil)- $\beta$ -naph- thoquinaldine methacetate	10	--	--	50	25	25	--	5
107	2(p-Dimethylamino anil)-4-ethoxy- $\alpha$ -naph- thoquinaldine methosulphate	25	--	--	25	25	10	--	50
108	Quinosol	50	50	50	100	100	100	25	50

\*The figures represent thousands.

ing it ineffective, and (3) the serum acting as protector altering the surface of the bacteria, making them less permeable to the dye.

#### BACTERICIDAL TEST

A departure from conventional bactericidal methods was adopted in this study. The same gram-positive and gram-negative organisms used in the preceding experiment were tested. As in that experiment 10 per cent serum pneumococcus broth medium of pH 7.6 was used. Culture tubes containing 5 c.c. of this broth were inoculated with 0.1 c.c. of eighteen-hour broth culture and incubated at 37.5° C. for two hours. As most organisms have a lag period of from one to three hours, it seemed advisable to add the compound which was to be investigated after this lag period, thus giving the advantage to the organism and not to the dye. After two hours of incubation of series of tubes,

various amounts of the dye were added, making a dilution of 25,000 to 200,000 for the gram-positive, and for the gram-negative organisms dilutions were from 5,000 to 100,000. The tubes were well shaken and returned for further incubation and readings made at the twenty-fourth hour. At the forty-eighth hour, subinoculations were made from each tube on serum agar plates in order to rule out the possibility of bacteriostasis, which occurs in such experiments after a tube appears to be perfectly clear, when if subcultured colonies develop. To conserve culture media a number of streaks were made on each plate using the method described elsewhere.

The end-results were recorded after the plates had been incubated for forty-eight hours.

The final results were disappointing. The values of the bactericidal activities, of all the compounds used in the bacteriostatic test and summarized in Tables I, II, and III, were approximately one-half and occasionally one-third of those obtained in the bacteriostatic test. The summaries of the tables are omitted as the findings are of no importance.

These studies reveal that the streptococci as a group are much more resistant to bacteriostasis than other gram-positive organisms. *Streptococcus faecalis* is the most resistant, *viridans* is next in order, followed by *nonhemolyticus*, *scarlatina* and *erysipelatis*. *Streptococcus hemolyticus*, on the other hand, is much more sensitive to the dyes. Next to the streptococci is *Staphylococcus P. aureus*. Pneumococci are more sensitive to bacteriostasis than any of the gram-positive organisms studied. Type II is the most sensitive, type III follows closely and type I reacts less than the others.

Of the gram-negative organisms, the three dysentery organisms studied possessed about similar sensitivities to bacteriostasis. *Bibrio comma* and *B. typhosus* follow very closely. The *B. paratyphosus* A and B and *B. coli* are extremely resistant to the action of the dyes.

During the course of these studies casual observations were made on the comparison of the appearance of the growth in the dye compartment with the growth of the control. Repeatedly we noticed that some dyes exerted only a slight bacteriostatic action—the growth was scanty, here and there sprinkled with single colonies which varied in topography and other physical properties. This was especially pronounced in media containing the dyes of the azine series. This is in accord with many observations reported in the past dealing with forced dissociation of bacteria, which have been fully discussed by Hadley.<sup>2</sup> It seems that, in the future, this phenomenon must be taken into consideration when selecting compounds for studies such as this. We see no reason why the growth should be our only criterion in the evaluation of a drug, and not the variants which are present in the growth.

#### CONCLUSIONS

In this first contribution, we have presented bacteriostatic and bactericidal studies of 130 dyes and allied compounds, most of which have, in our hands, proved to be of little or no value. The most effective for the gram-positive

organisms were: Groups V, triphenyl methane dyes (basic); VII, acridine dyes; IX, azine dyes, (a) chinoxalines, (b) safranines; X, oxazine dyes; XI, thiazine dyes; XIV, anil quinoline compounds; and a few of XVI, alkaloids and derivatives.

The gram-negative organisms are affected differently. Group V, triphenyl methane dyes (basic), iodine green, methyl green, brilliant green, and basic fuchsin have moderate bacteriostatic and bactericidal properties, and VII, acridine dyes, only feeble activities. Anil quinoline compounds, Group XIV, are the most effective.

At present the most promising is Group XIV, anil quinoline compounds. Many new modifications with and without mercury have been prepared in this laboratory, and the results will be reported later.

#### REFERENCES

1. Churchman, J. W.: The Selective Bactericidal Action of Gentian Violet, *J. Exper. Med.* 16: 221, 1912.
2. Hadley, P.: Microbic Dissociation, *J. Infect. Dis.* 40: 1, 1927.

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## THE BLOOD WASSERMANN TEST IN 500 CASES OF NEUROSYPHILIS WITH POSITIVE CEREBROSPINAL FLUIDS\*

WILLIAM C. MENNINGER, M.D., TOPEKA, KAN., AND  
LEON BROMBERG, M.D., CHICAGO, ILL.

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ALTHOUGH many syphilologists and neurologists have expressed the opinion that a negative blood Wassermann frequently is found in neurosyphilis, there have been relatively few clinical studies to support this statement. We here present the results of an investigation of 500 cases of neurosyphilis, chosen on the basis of a positive cerebrospinal fluid in addition to clinical studies which established the diagnosis, as well as the active state of the neurosyphilitic process. In reviewing unselected cases from our record files over a period covering the past eight years, approximately 20 patients with negative cerebrospinal fluids were excluded despite sufficient clinical evidence of syphilis of the central nervous system. This study shows the frequency of a negative, doubtful, and strongly positive blood Wassermann test in the five more common clinical forms of neurosyphilis, all of which showed a positive Wassermann test in the spinal fluid: asymptomatic (297 cases), tabes dorsalis (133 cases), general paralysis (38 cases), taboparesis (19 cases), and meningovascular (13 cases).

The statistical data which are analyzed later were based upon the initial blood and spinal fluid tests taken early during the patient's study in the clinic.

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\*From the Public Health Institute, Chicago.  
Received for publication, July 17, 1934.



Only those cases in which the two tests were taken approximately at the same time were included in this series. Notation was made of the amount of treatment the patient had had previous to the time of the lumbar puncture. In subsequent tables the treated and untreated cases are separately considered under their proper headings.

For the past three years (which time interval includes most of the present series of 500 cases), we have employed the standard Kolmer technic for the examination of both blood and spinal fluid. Prior to that time a less sensitive technic was used, but since both the blood and spinal fluid were examined by the same method, the validity of comparative results is not impaired.

The routine study of the spinal fluid has also included a cell count, tests for globulin, and colloidal gold curve determination. Since these latter results, in general, corresponded to the degree of positivity of the Wassermann test in the spinal fluid, they have not been analyzed in this paper.

#### ASYMPTOMATIC NEUROSYPHILIS

Our study includes 297 cases so classified, all with four-plus cerebrospinal fluid Wassermann tests (Table I).

TABLE I

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT OF ALL 297 CASES	PATIENTS WITHOUT TREATMENT (BOTH MALE AND FEMALE)	PER CENT OF 83 UNTREATED PATIENTS
Negative	85	16	101	33.6	16	19.2
One-plus	5	4	9	3.0	0	0.0
Two-plus	29	10	39	14.0	10	12.2
Three-plus	22	4	26	8.4	7	8.4
Four-plus	74	48	122	41.0	50	60.2
Totals	215	82	297	100.0	83	100.0

Thus, we find that one-third of this large series of cases of asymptomatic neurosyphilis, on their initial examination in our clinic showed a negative blood Wassermann test in the presence of a positive cerebrospinal fluid Wassermann test. An additional nine cases (3.0 per cent) showed only a one-plus blood Wassermann test. Even if one granted that previous treatment might be responsible for the blood Wassermann reversal in a large percentage of cases, it is noteworthy that 19.2 per cent of the 83 patients who gave no history of treatment also showed a negative blood Wassermann test on the initial examination.

The opinions and findings of other workers as to the frequency of a negative blood Wassermann test in asymptomatic neurosyphilis, is shown in Table II.

#### TABES DORSALIS

The findings in 133 cases of tabes dorsalis, in which every case showed a four-plus Wassermann test in the cerebrospinal fluid are shown in Table III.

It is worthy of comment that 27.8 per cent of all these patients had a negative blood Wassermann on admission, and an additional 7.5 per cent showed an equivocal reaction. Even 21.3 per cent of the untreated patients showed a negative or doubtful blood finding, though all showed clinical evidence of *tabes dorsalis* as well as strongly positive spinal fluid Wassermann tests.

That *tabes dorsalis* is frequently associated with a negative blood Wassermann is shown in the uniformity of the reports and opinions of previous investigators (Table IV).

TABLE II

AUTHOR	YEAR	FINDINGS
Fildes, Parnell, Maitland <sup>1</sup>	1919	624 patients, 2 with negative blood in 42 patients with positive spinal fluid
Fordyce and Rosen <sup>2</sup>	1921	14 per cent of late asymptomatic neurosyphilis with negative blood and positive fluid
Wörster-Drought, et al. <sup>3</sup>	1922	14 patients, 12 with negative blood
Fuchs <sup>4</sup>	1922	131 patients, 29 per cent with negative blood
Stokes and Brown <sup>5</sup>	1922	112 patients, 72 (69.2 per cent) with positive fluid and negative blood
Stokes <sup>6</sup>	1926	30 to 50 per cent negative blood in latent early neurosyphilis
Moore <sup>7</sup>	1933	"Blood is often reversed and maintained as negative"

TABLE III

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT OF SERIES OF 133 TABETICS	PATIENTS WITHOUT TREATMENT (BOTH SEXES)	PER CENT OF 47 UNTREATED TABETICS
Negative	36	1	37	27.8	9	19.2
One-plus	10	0	10	7.5	1	2.1
Two-plus	13	2	15	11.3	6	12.8
Three-plus	11	0	11	8.2	7	15.1
Four-plus	53	7	60	45.2	24	50.8
Totals	123	10	133	100.0	47	100.0

TABLE IV

AUTHOR	YEAR	FINDINGS
Schaffers <sup>8</sup>	1911	150 cases, negative blood in 31.3 per cent
Nonne <sup>9</sup>	1916	30 to 40 per cent with negative blood
Fordyce <sup>10</sup>	1919	21 cases, 19 per cent with negative blood; 100 per cent had positive spinal fluid
Boyd <sup>11</sup>	1920	30 to 50 per cent with negative blood. Spinal fluid also negative in the same per cent
Gennerich <sup>12</sup>	1921	30 to 40 per cent negative blood
Wörster-Drought, et al. <sup>3</sup>	1922	36 cases, five with negative blood
Thaysen <sup>13</sup>	1923	111 cases, 46 with negative blood (42 per cent)
Stokes and Shaffer <sup>14</sup>	1925	30 to 60 per cent with negative blood
Kilduffe <sup>15</sup>	1926	30 per cent negative blood; spinal fluid 100 per cent positive
Fritzschke <sup>16</sup>	1928	15.4 per cent of cases with negative blood and positive fluid
Plaut <sup>17</sup>	1929	20 to 30 per cent negative blood
Geert-Jørgensen, et al. <sup>18</sup>	1932	216 cases, 50 (23.1 per cent) with negative blood and positive fluid
Schamberg and Wright <sup>19</sup>	1932	35 per cent negative blood serum

## GENERAL PARALYSIS

The blood Wassermann findings in 38 cases of clinical and seropositive spinal fluid cases of general paralysis are shown in Table V.

TABLE V

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT	CASES WITHOUT TREATMENT (BOTH SEXES)
Negative	6	2	8	21.1	1
One-plus	1	0	1	2.6	0
Two-plus	5	0	5	13.1	0
Three-plus	4	0	4	10.5	0
Four-plus	12	8	20	52.7	6
Totals	28	10	38	100.0	7

In this series we found that 21.1 per cent of cases showing both the clinical picture of general paralysis, as well as positive spinal fluid findings, had a negative blood Wassermann test. Admittedly the number of cases analyzed is too small to warrant any definite conclusion and the series could not be fairly compared with similar studies made on patients suffering from general paralysis whose mental symptoms were severe enough to demand hospitaliza-

TABLE VI

AUTHOR	YEAR	FINDINGS
Marie and Levaditi <sup>20</sup>	1907	39 cases, 41 per cent negative blood and 27 per cent negative spinal fluid
Edel <sup>21</sup>	1908	62 cases, 100 per cent positive blood
Lesser <sup>22</sup>	1908	62 cases, 100 per cent positive blood
Raviart, Breton, Petit <sup>23</sup>	1908	72 cases, 7 per cent negative blood
Stertz <sup>24</sup>	1908	"95 per cent positive fluid and serum even higher percentage positive"
Marinescu <sup>25</sup>	1909	35 cases, 6 per cent negative blood
Nonne and Holzmann <sup>26</sup>	1910	100 per cent positive blood
Darling and Newcomb <sup>27</sup>	1916	69 cases, fluid 100 per cent positive, blood negative in 8 (11.6 per cent) cases
Fordyce <sup>10</sup>	1919	40 cases, 5 per cent negative blood, 100 per cent positive fluids
Plaut <sup>28</sup>	1920	1,400 cases, 8 negative blood, and 14 with weakly positive blood
Kafka <sup>29</sup>	1920	100 cases, 24 with negative blood
Craig <sup>30</sup>	1921	100 per cent positive blood
Nathan and Weichbrodt <sup>31</sup>	1921	150 cases, 27 with negative blood at some stage
Boas <sup>32</sup>	1922	243 cases, 100 per cent positive blood, 94 per cent positive fluid
Eicke <sup>33</sup>	1922	110 cases, 8 with negative blood
Eskuchen <sup>34</sup>	1923	72 cases, 5.5 per cent with negative blood and positive fluid
Menninger and Menninger <sup>35</sup>	1925	166 cases, 7.6 per cent with negative blood and positive fluid
Walther and Abelin <sup>36</sup>	1926	32 cases, 3 with negative blood
Kilduffe <sup>15</sup>	1926	100 per cent positive blood
Fritzsche <sup>16</sup>	1928	4.3 per cent with positive fluid and negative blood
von Rohden, et al. <sup>37</sup>	1931	437 cases, 10 untreated had negative blood
Candler and Maun <sup>38</sup>	1932	173 cases, 11 with positive fluid and negative blood
Peterson <sup>39</sup>	1932	179 cases, 18.5 per cent with negative blood
Salomon <sup>40</sup>	1932	120 cases, 13 with negative blood
Solomon <sup>41</sup>	1932	5 to 10 per cent with negative blood

tion. However, the series is sufficiently large to represent a cross-section of the average experience in private or out-patient clinic practice.

Even though general paralysis has been a special field of investigation as to the relationship of blood and spinal fluid findings, there still remains a wide discrepancy of opinion, as shown in Table VI.

Since the improvement in the technique of the Wassermann test, it is not possible to compare the earlier reports with later studies, and the results since 1920 are probably much more accurate than those published in previous years.

The spinal fluid Wassermann reaction is said by many investigators to be positive in 100 per cent of cases of general paralysis (Eichelberg,<sup>42</sup> Darling and Newcomb,<sup>27</sup> Sabatini,<sup>43</sup> Boyd,<sup>44</sup> Hagnenau,<sup>44</sup> Sicard,<sup>45</sup> Cestan and Riser,<sup>46</sup> Nonne,<sup>9</sup> Fordyce,<sup>10</sup> Craig<sup>30</sup>). As is indicated in Table VI, however, there have been many cases with a completely negative spinal fluid reported by reliable workers over a period of years. Furthermore, there have been many unquestioned cases of other types of neurosyphilis reported showing a negative spinal fluid. A classification of such cases was made by Nonne<sup>17</sup> and subsequently expanded by Solomon and Klander.<sup>47</sup> It has been conclusively shown by Chesney and Kemp,<sup>49</sup> that the *Spirocheta pallida* can be cultivated from the spinal fluid in 14.7 to 26.6 per cent of cases of early syphilis without changes of any kind in the spinal fluid.

#### TABOPARESIS

Table VII presents the findings of the blood Wassermann test in 19 cases of seropositive spinal fluid cases of taboparesis.

TABLE VII

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT	PATIENTS WITHOUT TREATMENT (BOTH SEXES)
Negative	5	0	5	26.3	1
One-plus	1	0	1	5.2	0
Two-plus	1	0	1	5.2	1
Three-plus	1	0	1	5.2	0
Four-plus	10	1	11	58.1	2
Totals	18	1	19	100.0	4

This survey shows that 26.3 per cent of the 19 patients had a negative blood Wassermann test on their admission to the clinic. Again the number of cases is too small to permit any very definite statement as to the frequency with which a negative blood Wassermann is encountered in this condition. The figures do demonstrate, however, the fact that in dealing with a non-hospitalized group of patients with this type of neurosyphilis, a comparatively large percentage will present a negative blood Wassermann test.

There has been little specific mention of the laboratory findings in taboparesis in the literature. Worster-Drought et al<sup>3</sup> (1922) reported nine cases of taboparesis, four of which had negative blood Wassermann tests. Kilduffe<sup>15</sup> (1926) stated that the blood and spinal fluid are 100 per cent positive in taboparesis.

MENINGOVASCULAR NEUROSYPHILIS

The findings in 13 cases of meningovascular neurosyphilis, all with positive evidence of cerebral vascular damage and with a positive spinal fluid, are shown in Table VIII.

TABLE VIII

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT	PATIENTS WITHOUT TREATMENT
Negative	2	2	4	30.8	0
One-plus	0	0	0	0.0	0
Two-plus	1	1	2	15.4	0
Three-plus	0	0	0	0.0	0
Four-plus	5	2	7	53.8	1
Totals	8	5	13	100.0	1

The series presented is obviously too small to allow any doctrinal conclusions, but the findings remain consistent with those of the other clinical types in showing a surprisingly large percentage of cases with negative blood tests.

We have found no definite reference to the laboratory findings in meningovascular syphilis in the literature. This is probably explained on the basis of varying diagnostic categories, and such cases may have elsewhere been included in the cerebrospinal, the meningitic, or the vascular groups.

FIVE HUNDRED CASES OF NEUROSYPHILIS

The findings for the entire series are shown in Table IX.

TABLE IX

BLOOD SERUM WASSERMANN	MALES	FEMALES	TOTAL	PER CENT OF ALL 500 CASES	PATIENTS WITHOUT TREATMENT (MALE AND FEMALE)	PER CENT OF 142 UNTREATED CASES
Negative	134	21	155	31.0	27	19.0
One-plus	17	4	21	4.2	1	0.7
Two-plus	49	13	62	12.4	17	12.0
Three-plus	38	4	42	8.4	14	9.9
Four-plus	154	66	220	44.0	83	58.4
Totals	392	108	500	100.0	142	100.0

The advantage in grouping all the various clinical types of neurosyphilis in the above table is briefly to demonstrate the remarkable fact that 31 per cent of all such cases presented a negative Wassermann test in the blood, although showing a positive test in the spinal fluid, commonly accepted as evidence of active neurosyphilis. Of the 142 patients without a history of any previous antisyphilitic treatment, a surprisingly high percentage (19.0) showed a negative blood Wassermann test. This might logically lead us to assume that the amount of treatment received might account for about 12 per cent of the negative blood Wassermann tests in the group as a whole. This is probably fallacious, however, since the history in some cases was so vague as to amount and kind of therapy that little dependance could be placed upon

it. Furthermore, a considerable number of patients included in the treated group had previously received such desultory and ineffective treatment as a few mercuric inunctions or two or three intravenous injections.

There have been few studies of large groups of neurosyphilis patients reported in the literature with special reference to the blood and spinal fluid laboratory findings. Numerous reports divide their cases into the various diagnostic categories and have been listed in the previous tables. Table X gives studies of neurosyphilis as a group.

TABLE X

AUTHOR	YEAR	FINDINGS
Eskuchen <sup>34</sup>	1923	346 cases, 97 (28.0 per cent) with negative blood and positive spinal fluid
Dattner <sup>50</sup>	1932	900 cases, 20 per cent with negative blood
Haag and Kolbe <sup>51</sup>	1932	321 cases, 18 per cent with positive fluid and negative blood
Geert-Jørgensen, et al. <sup>18</sup>	1933	654 cases, 94 (14.3 per cent) with negative blood and positive fluid

## THE KAHN TEST

The Kahn test was routinely made on the blood in each of our cases. It is generally recognized that the Kahn test is more sensitive than the Wassermann test, and hence it is of interest to compare the findings of the Kahn test in the cases with a negative blood Wassermann test in this series. In the 155 cases with a negative blood Wassermann, the blood Kahn was positive in 38 cases. In these 38 instances the Kahn test was "two-plus" in six cases, "three-plus" in 7 cases, and "four-plus" in 25 cases.

Of the entire 500 cases studied, 117, or 23.4 per cent, showed completely negative blood tests, both Wassermann and Kahn.

## THE CEREBROSPINAL FLUID WASSERMANN TEST

As previously stated, the present study includes only patients with a positive spinal fluid Wassermann test. An analysis of the titer of fluid positivity discloses the following: of the total of 500 cases 423 showed a four-plus spinal fluid Wassermann reaction with as little as 0.2 c.c. of fluid; of the remaining 77 patients 27 showed a four-plus reaction in the spinal fluid Wassermann with 0.4 c.c. fluid; in 39 cases 0.6 c.c. was the least amount of fluid to show a completely positive Wassermann test; in 11 cases 1 c.c. of spinal fluid was required for a four-plus Wassermann test. It is interesting to correlate these findings with the Wassermann test performed on the blood serum, as shown in Table XI.

TABLE XI

BLOOD SERUM WASSERMANN	SPINAL FLUID FOUR-PLUS IN:		
	0.4 c.c.	0.6 c.c.	1.0 c.c.
Negative	11 cases	20 cases	6 cases
One-plus	2 cases	1 case	0 case
Two-plus	7 cases	1 case	1 case
Three-plus	0 case	2 cases	0 case
Four-plus	7 cases	9 cases	4 cases
Totals	27 cases	39 cases	11 cases

## SUMMARY

The problem of the status of the blood Wassermann in 500 cases of clinical neurosyphilis with positive spinal fluids is presented, together with a brief review of the literature.

1. In the entire group of all clinical types of neurosyphilis, 155 (31 per cent) showed negative blood Wassermann tests early in their study at the clinic. Twenty-one patients (4.2 per cent) showed a one-plus blood Wassermann test; 62 patients (12.4 per cent) showed two-plus; 42 patients (8.4 per cent) showed three-plus; and 220 patients (44 per cent) showed a four-plus blood Wassermann test.

2. In 297 cases of asymptomatic neurosyphilis, 101 patients (33.6 per cent) showed a negative blood Wassermann test.

3. In 133 cases of *tabes dorsalis*, 37 patients (27.8 per cent) showed a negative blood Wassermann test.

4. In 38 cases of general paralysis, 8 patients (21.1 per cent) showed a negative blood Wassermann test.

5. In 19 cases of *taboparesis*, 5 patients (26.3 per cent) showed a negative blood Wassermann test.

6. In 13 cases of meningovascular neurosyphilis, 4 patients (30.8 per cent) showed a negative blood Wassermann test.

7. The Kahn test was positive in 38 cases showing a negative blood Wassermann.

If this number of cases is deducted from the 155 showing a negative blood Wassermann, there remain 117 patients (23.4 per cent) with both the Kahn and Wassermann tests in the blood negative.

## CONCLUSIONS

1. In approximately 30 per cent of cases of active neurosyphilis with positive spinal fluid tests, the blood serum Wassermann test fails to give any indication of this process. In 23 per cent neither the blood Kahn nor Wassermann test is positive.

2. In view of this fact, no case of syphilis, after the early stage, can be regarded as completely studied, accurately diagnosed, or correctly treated, without knowledge of the cerebrospinal fluid findings.

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# DIURNAL VARIATIONS IN CONCENTRATION OF RED BLOOD CELLS AND HEMOGLOBIN\*

JAMES J. SHORT, M.D., NEW YORK, N. Y.

NUMEROUS data have accumulated over many years on the subject of red cell and hemoglobin values in the peripheral blood stream. Despite earnest and painstaking efforts on the part of many competent workers, the question as to whether significant physiologic diurnal variations occur is still in the realm of controversy. In 1923, Rabinowitch<sup>1</sup> reported hemoglobin variations of from 1.1 to 26.2 per cent, within a period of ten hours during the day. In a later report Rabinowitch and Strean<sup>2</sup> concluded from further studies that hemoglobin is distributed on the surface of the cell stroma and that corpuscular amount of hemoglobin is proportionate to the cell surface.

Barcroft and coworkers<sup>3</sup> presented data to indicate that the spleen may act as a reservoir for red cells from which the circulating cells may receive additional recruits to meet certain exigencies. Osgood and Haskins<sup>4</sup> refer to spontaneous diurnal variations of red cells and hemoglobin but feel that further work should be done on this subject.

We are presenting herewith some carefully conducted observations† bearing on diurnal fluctuations in normal individuals. Those studied were going about their daily duties which for the most part were of a clerical nature. A few studies were made on the effect of sudden active exercise. Results are reported in Tables II and III. No attempt has been made to review the numerous opinions bearing on this subject. This has been exhaustively done by Smith<sup>5</sup> to whose article the reader is referred.

## TECHNIC

Duplicate counts were made on each specimen with the use of a separate pipette for each count. Similar duplicate pipettings were also used for the hemoglobin determinations. The erythrocyte pipettes were shaken in a mechanical shaker for several minutes to insure an even distribution of the cells. A Bausch and Lomb modified Bürker-Newbauer counting chamber was used and care was taken to obtain an even distribution of cells over its surface when a drop was applied. Technic was carried out by a highly competent and experienced technician with the exception of the earlier observations which were made by the author. It was felt that although both workers usually agree closely in hemoglobin readings and blood counts, the results would be more comparable in studying fluctuations if each series of observations were made by a single individual.

\*From the Laboratories of the Life Extension Institute, New York, and the Department of Medicine, New York Postgraduate Medical School of Columbia University.  
Received for publication, September 12, 1934.

†Complete tables of all experiments will appear in the author's reprints.

Blood for counts and hemoglobin estimations were taken by venipuncture, care being taken to avoid undue or prolonged stasis.

As a check upon the accuracy of the technic, blood specimens were also drawn from the veins of four individuals into dry Luer syringes and discharged immediately into bottles containing dry powdered oxalate; these were thoroughly shaken and submitted by number for erythrocyte counts and hemoglobin determinations. The technician had no knowledge of the identity of the specimens thus submitted. The results which were assembled later are shown in Table I. It will be seen from Table I that although the maximal variation in cell counts was 700,000 and in hemoglobin was 7 per cent, in general the average differences between average counts and hemoglobin readings were less than the findings in the study of diurnal variations. We feel justified, therefore, in believing that the diurnal variations noted in Table II are much greater than can be explained on the basis of technical error.

#### DISCUSSION

In the study of diurnal variations changes of from 1 to 17 per cent of hemoglobin and from 70,000 to 940,000 erythrocytes were observed within a period of eight hours with the subject at a moderate amount of physical activity; and variations of as much as 10 per cent of hemoglobin and 900,000 erythrocytes were observed within a few minutes following active exercise, such as stair running (see Table III). Erythrocytes and hemoglobin concentrations did not always parallel each other. This observation suggests the possibility that the hemoglobin content of the individual cell is not a fixed quantity but is subject to sudden changes, or that in responding to the demands of the organism, erythrocytes with different increments of hemoglobin are added to the circulation from some depot, such as the spleen<sup>3</sup> with sufficient rapidity to modify materially the quantities present in the peripheral circulation.

Krumbhaar<sup>6</sup> states that he has always felt that "a very annoying lack of correspondence between hemoglobin and red cell counts must be largely or entirely explained by the unavoidable margins of error in the technical method." He further states that this has bothered him in more than twenty years of anemia studies, and that he occasionally notes in the literature where others have attributed false significance to the apparently uncontrollable variations.

Smith<sup>5</sup> although admitting in her article that "some believe in a constant number (of red cells) and some in a widely varying one" gives as the result of her own extensive observations, the opinion that "within short periods of time, certainly the eight-hour periods investigated, the total red blood cell count is practically constant, and that any fluctuations present are within the limits of error of the method." She states further that "variations in hemoglobin determinations are similar and parallel to those of the total red counts," although "large diurnal variations have been found more frequently than small ones by previous investigators."

TABLE I  
SHOWING RESULTS OF ERYTHROCYTE COUNTS AND HEMOGLOBIN READINGS ON DIVIDED SPECIMENS. FINDINGS IN EACH CASE SHOULD AGREE AND VARIATIONS THEREFORE REPRESENT TECHNICAL ERROR

CASE	ERYTHROCYTE COUNTS				HEMOGLOBIN READINGS			
	TIMES BLOOD SPECIMEN DIVIDED	AVERAGE OF BLOOD COUNTS	MAXIMAL VARIATIONS		TIMES BLOOD SPECIMEN DIVIDED	AVERAGE OF HEMO- GLOBIN READINGS	MAXIMAL VARIATIONS	
			IN INDIVIDUAL BLOOD COUNTS	IN AVERAGE OF DUPLICATE COUNTS			IN INDIVIDUAL HEMO- GLOBIN READINGS	IN AVERAGE OF DUPLICATE READINGS
M. F. K. 6/18/34	4	mil. 4.740	mil. 0.290	mil. 0.215	4	gm. 12.775	gm. 0.5	gm. 0.35
A. R. G. 6/18/34	5	5.034	0.380	0.265	5	14.19	0.5	0.35
S. M. 6/25/34	5	4.980	0.280	0.195	10	14.17	1.3	0.8
F. P. D. 6/25/34	5	4.865	0.700	0.540	10	14.07	1.0	0.5
						per cent 93.25	per cent 4	per cent 2.5
						90.0	2	2.0
						89.85	7	3.0
						89.20	6	3.5

In the light of the present studies and previous observations, we do not believe that the lack of correspondence mentioned by Krumbhaar can be explained entirely on the basis of technical error. The independent fluctuations of red cells and hemoglobin over short periods of time are too great to be explained on this basis. We believe a better tentative explanation can be found in the work of Barcroft, or in the possible supposition that free hemoglobin may be taken up as such directly by the erythrocytes from certain depots, perhaps within the reticulo-endothelial system of the spleen or liver.

A few observations were made on the viscosity of blood before and after stair running by the method of Hess. With the Hess viscosimeter the rate of flow of freshly drawn blood through capillary tubes is compared with that of

TABLE II

SHOWING DIURNAL VARIATIONS IN CONCENTRATIONS OF RED CELLS AND HEMOGLOBIN, SUBJECTS ENGAGED IN SEDENTARY DUTIES. SPECIMENS TAKEN ON EACH DAY DURING WORKING HOURS AT APPROXIMATELY 9:00, 11:00, 1:00, 3:00, AND 5:00 O'CLOCK

CASE	SEX	AGE	DATE	ERYTHROCYTE COUNTS				HEMOGLOBIN READINGS			
				AVERAGE OF ALL COUNTS	COLOR INDEX	MAXIMAL DIURNAL VARIATION		AVERAGE OF ALL READINGS		MAXIMAL DIURNAL VARIATION	
						RED CELLS	COLOR INDEX				
A. R.	F	27	6/12/33	mil. 4.28	1.02	mil. 0.51	0.19	gm. 13.2	% 97	gm. 1.0	% 8
			6/20/33	4.28	1.00	0.07	0.04	13.1	95	0.7	5
			6/28/33	4.32	0.96	0.50	0.07	12.6	92	2.3	17
M. F. K.	F	42	6/14/33	5.08	0.96	0.66	0.09	14.8	109	0.7	5
			6/21/33	4.97	0.91	0.34	0.06	13.8	101	1.3	10
			6/27/33	4.93	0.98	0.39	0.05	14.7	107	1.0	7
F. P. D.	M	25	5/ 3/33	4.64	0.96	0.60	0.07	13.8	88	1.2	7
			5/10/33	4.39	0.96	0.31	0.08	13.4	85	0.7	5
			5/17/33	4.49	0.95	0.30	0.05	13.4	85	1.5	10
B. R.	M	18	5/19/33	5.03	0.90	0.42	0.05	14.2	90	1.8	11
			5/26/33	4.84	0.88	0.10	0.09	13.4	85	1.0	7
			6/ 2/33	4.54	0.92	0.43	0.07	13.2	84	1.5	10
J. J. S.	M	28	10/10/24	4.82	0.93	0.50	0.08	13.9	88	0.5	3
E. H.	F	32	10/ 7/24	4.29	0.88	0.94	0.17	11.4	83	0.4	3
			10/10/24	4.36	0.88	0.43	0.08	11.6	85	0.1	1
M.	F	30	9/17/24	4.72	0.89	0.63	0.13	12.8	93	0.7	5
M.	F	30	9/ 2/24	4.35	0.83	0.42	0.11	10.9	80	0.8	5
R. F.	F	41	5/24/33	4.42	1.01	0.61	0.13	13.5	99	1.2	9
			5/31/33	4.57	1.01	0.28	0.06	14.1	103	0.5	3
			6/ 7/33	4.34	1.01	0.36	0.11	13.3	98	1.4	11
A. R. G.	M	27	4/29/33	4.62	1.03	0.74	0.09	14.8	94	1.7	10
			5/ 4/33	4.92	0.95	0.42	0.19	14.6	92	1.4	9
			5/12/33	4.90	0.98	0.34	0.07	15.0	95	0.8	5
A. G.	M	25	9/28/24	3.30	1.38	0.84	0.34	13.9	88	0.5	3
H. J.	M	32	5/ 8/33	5.87	0.87	0.18	0.06	15.9	101	0.7	5
			5/15/33	5.78	0.88	0.42	0.11	15.9	101	1.2	8

distilled water when both are subjected to the same pressure. The figures express the ratio of the length of the column of water to that of the column of blood, the latter being taken as unity. Thus high figures represent high viscosity and vice versa. Viscosity of the blood is due chiefly to the concentration of the suspended elements and represents internal friction between

TABLE III

EFFECT OF EXERCISE ON RED CELLS, HEMOGLOBIN, BLOOD VISCOSITY AND CELL VOLUME

CASE	SEX	TIME	R.B.C. MILLIONS PER C.M.M.	HEMO.		COLOR INDEX	VIS- COSITY	CELL VOLUME %
				%*	GM.			
J. J. S.	M	Control	4.49	98	15.5	1.11	4.9	35.3
		Exercise (stair running) for one minute						
		11:00	4.82	108	17.0	1.13	5.8	40.3
		11:15	5.39	97	15.3	0.91	5.1	37.3
		11:33	5.20	94	14.8	0.90	5.1	33.8
M. M.	M	Control	3.70				4.6	37.8
		Exercise (stair running) for one minute						
		11:10	4.58				5.2	41.2
		11:25	4.22				4.7	42.0
		11:40	4.36				4.6	39.1
J.	F	Control	4.39				4.5	37.1
		Exercise (stair running) for one minute						
		11:49	3.90				4.7	38.0
		11:51	3.90				4.5	37.6
		12:21	3.63				4.4	36.6
A. W.	F	Control	4.73	82	11.2	0.78		
		Exercise (stair running) for two minutes						
J. G.	M		5.04	84	11.5	0.75		
		Control	5.41	91	14.4	0.84		
		Exercise (stair running) for two minutes						
R. P.	F		5.97	86	13.6	0.73		
		Control	4.81	84	11.5	0.79		
		Exercise (stair running) for two minutes						
			5.37	94	12.9	0.79		

\*100 per cent: male 15.8 gm., female 13.7 gm., per 100 c.c.

these elements. Therefore an increase in the number of cells or of their average size will increase viscosity. It has long been known that there is an increase in the size of individual cells as a result of an increased increment of carbon dioxide and probably also of lactic acid. In a recent monograph on red blood cell diameters,<sup>7</sup> while discussing their diurnal variation, Cecil Price-Jones states that although gentle exercise has no special influence, violent exercise such as stair running increases the diameters. His observations show increases in the average diameter of individual cells of from 0.16 to 0.6  $\mu$  or 2.2 to 8.4 per cent. These changes he attributes to increased amounts of CO<sub>2</sub> and lactic acid in the blood stream, since it has long ago been shown by Gürber,<sup>8</sup> von Limbeck<sup>9</sup> and by Hamburger<sup>10</sup> that the addition of CO<sub>2</sub> to blood increased the volume of the red cells. Ryffel<sup>11</sup> showed also that there is a considerable increase in the lactic acid in the blood at the end of short violent exercise, but not with moderate exercise of longer duration. So it would be expected that viscosity would increase following sudden exercise, and this phenomenon was shown in the few observations made. An increase of cell volume was also shown and was to be expected.

It should be noted that in two glucose tolerance tests in which hemoglobin studies were made, there was no significant variation of the hemoglobin concentration during the two-and-one-half-hour period of each test. This would seem to indicate that blood dilution was not sufficient following the ingestion of 100 gm. of glucose in 50 per cent solution to change the hemoglobin values.

#### SUMMARY AND CONCLUSIONS

1. Spontaneous variations in red cell counts up to 940,000 per c.mm. and in hemoglobin values up to 17 per cent were observed in normal individuals going about their usual sedentary occupations within a period of eight hours. Less marked but sudden changes were observed in those subjected to active exercise such as stair running.

2. Hemoglobin and red cell concentrations did not always vary in the same direction at the same time. This suggests that hemoglobin either is not a fixed quantity in the individual cells but is subject to variation, or that new red cell recruits with different increments of hemoglobin are added to the blood stream from time to time with sufficient rapidity to modify former values.

3. A few viscosity and cell volume determinations were made at intervals before and after exercise. The increase in both is explained by the greater number of red cells in circulation and a probable increase in the individual cell volume as a result of the circulation of larger amounts of carbon dioxide and lactic acid.

4. In two glucose tolerance tests, hemoglobin determinations were made on the bloods over two-and-one-half-hour periods and no significant variations were found. This would tend to indicate that blood dilution was not sufficient to produce appreciable variations in hemoglobin concentrations.

5. The chief purpose of this contribution is to add further evidence to the view widely held that rapid spontaneous variations in red cells and hemoglobin can occur within short intervals and that red cell and hemoglobin values do not necessarily parallel each other.

The author wishes to acknowledge the assistance of Frank P. Dunbar who did much of the technical work recorded in this article.

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## THE LABORATORY AS AN APPROACH TO ANEMIC THERAPY\*

TRUMAN G. SCHNABEL, M.D., PHILADELPHIA, PA.

CIRCULATING erythrocytes are fundamentally responsible for the clinical pictures of the anemias. In anemic states the number of these cells is generally less than low normal, and frequently they present abnormal physical and chemical characteristics. Physical variations from the normal reveal themselves in shape, size, and lack of maturity. Concerning the chemical variations, it is not possible to be explicit to the same degree, since exact knowledge of the chemical constituents of the erythrocyte is almost limited to hemoglobin. The practical result, for the present, is that the outstanding abnormal chemical finding in anemia consists in a quantitative diminution of this important component of the red cell. Variations from the normal are due either to a loss of red cells or to a quantitative or qualitative failure of erythrogenesis. The loss of blood may result from a vascular leak or may be accounted for by an accelerated intravascular erythrocytic destruction. Unsatisfactory erythrogenesis may be consequent upon a number of unfavorable circumstances which influence erythropoiesis and the delivery of mature red cells to the peripheral circulation. Among these are certain congenital, nutritional and unknown factor deficiencies, the traumatism of chemical and bacterial poisons and the compression effects of neoplasms and excessive leucocytic proliferation in the leucoses.

While all of this is appreciated, we continue to meet the general assumption that the classification of anemias as primary and secondary is dependent upon the existence of a known or unknown cause and perhaps upon the type of color index. All the while, however, it is evident that a primary anemia may have a known cause and that an anemia regarded as secondary may have a wholly unknown cause. In this connection, reference can be made to the primary type of anemia observed in some patients with *Dibothriocephalus latus* infestation and to the secondary type of simple hypochromic anemia with no evident cause. As it develops, all anemias are secondary and any of the etiologic factors mentioned heretofore, whether wholly understood or not, may be responsible for an anemia. The same apparent cause, moreover, and this is more important, may be responsible for different varieties of anemias. These various types are defined by the number per unit volume of blood, the size and the pigment content of red cells so far as each of these attributes deviates from the normal, or so far as they vary from the normal in quantitative relationship to each other. On such a basis, we find that a single etiologic factor, pregnancy for example, may account for varied types of anemia. That an individual is anemic may be more

\*From the Department of Medicine, University of Pennsylvania.

Received for publication, August 2, 1931.

Read at the Meeting of the American Therapeutic Society, June 9, 1934.



apparent than real; decisive determination can be made only by virtue of laboratory procedures. These can, at the same time, be utilized to place an anemia among the several varieties.

It has not been many years since we were content, in our patient study, with an enumeration of red and white cells per cubic millimeter of blood, some rather unreliable estimations of its hemoglobin content and perhaps a differential leucocytic count. It is still of major importance to count the erythrocytes of suspected anemias. It is of equal importance to estimate the hemoglobin content of the blood. Parenthetically, it is preferable to make such determinations as grams per 10 c.c. of blood rather than percentage of the supposed normal. It must, however, from what has been said, be evident that determinations of the size of the red cells are needed before one can have an intelligent idea of the nature of an anemia and, more important, before one can apply a rational therapy. Erythrocytic size can be determined from a Price-Jones curve which visualizes the diameters and anisocytotic range of several hundred red cells, or from a hematocrit estimation of cell volume. Haden has recently reemphasized the value of the latter method, giving original credit to Capps. Haden makes use of an ordinary accurately calibrated centrifuge tube instead of an especially made hematocrit. This method seems easier and less time consuming; it may, moreover, detect changes which are not made evident by the Price-Jones technic.

For the purpose of classifying anemias these reports are utilized to calculate corpuscular constants or indices. The former include estimations of mean corpuscular volume, hemoglobin, and hemoglobin concentration, expressed as absolute figures. These are used by way of comparison with figures established by examination of a series of normals. The indices are calculated on a basis of comparison with ideal normals. Thus the volume index is expressed by volume per cent over red cell percentage when five million is counted as a normal number of cells and 46 is the volume coefficient, or the number of cubic centimeters of packed cells per five million cells per hundred cubic centimeters of blood. The normal limits of the volume index are 0.90 to 1.10. The color index is expressed as the result of dividing the hemoglobin percentage by the red cell percentage. Here, too, 100 per cent is counted as the ideal hemoglobin content and five million as the normal red cell count. The normal limits of color index are 0.90 to 1.10. The saturation index is determined by dividing the per cent of hemoglobin by the volume per cent. The normal limits are from 0.90 to 1.10. The indices of volume and saturation are the more valuable for practical purposes although the color index has been popular for many years. It is quite true that the constant figures may be more accurate as working guides, as pointed out by Clarke at the Philadelphia General Hospital, but the determination of indices seems a bit more convenient since it links present methods with those of the past. In any event, if the determination of indices leaves one in doubt, there is the possibility of resorting to a comparative consideration of the corpuscular constant values. From studies of anemic blood in this fashion, it has been demonstrated that red cells may be excessive,

normal or decreased in number, large, normal, or small in size and of increased, normal or diminished hemoglobin content. With three variables, one may have variable combinations and so it happens that Haden was able to point out that one may, from the laboratory point of view, have ten different types of anemia. For the present at least, as Wintrobe and others have found, only four of these types are outstanding in practice. They are all hypocythemic in type, but they differ in cellular size and in pigment content.

Of these different anemias, there is first of all the large celled or macrocytic group with a volume index greater than 1.1 or a mean corpuscular volume ranging from 95 to 160 cubic microns, a color index greater than 1.1 or a mean corpuscular hemoglobin of 30 to 52 micromicrograms and a normal saturation index or a mean corpuscular hemoglobin concentration of 31 to 38 per cent. Macrocytosis is produced from the megaloblastic level as Doan, Cunningham and Sabin put it, and it is believed that macrocytosis points very clearly to the lack of a maturation stimulus. When a patient presents a macrocytic anemia, this stimulus must be supplied by the use of an anti-anemic principle, found in a number of substances. We, therefore, prescribe whole liver, the fraction A, desiccated stomach, combinations of liver and stomach preparations, a diet after the directions given by Minot and Murphy in 1926 as being particularly rich in its vitamin content, or some especial preparation of Vitamin B. English hematologists are using marmite, a prepared extract of yeast flavored with celery seeds. Such antianemic preparations must certainly be potent and must be given in doses of adequate size. The laboratory indication that the dosage is adequate lies in an increase in the reticuloocyte and erythrocyte count after stipulated periods of administration. During satisfactory treatment the volume index approaches the normal level. In this connection, it must be remembered that during infectious attacks, in the presence of old age, arteriosclerosis, and central nervous lesions, dosage must be doubled or even tripled over the usual amounts. In this respect antianemic therapy is similar to that of insulin. Intramuscular and intravenous methods are more efficacious in the case of patients who are gravely ill, who manifest severe complications, or in whom the gastrointestinal tract does not accept or take up anti-anemic substances. This group of large celled anemias, referred to in the current literature as pernicious anemia and the allied macrocytic anemias, includes, besides the Addisonian variety of anemia, some anemias occurring in sprue, in *Dibothriocephalus latus* infestation, in pregnancy, and in the macrocytic anemias of the tropics. Very occasionally, also, we find this same variety of anemia occurring after intestinal stricture and resection, with fatty chylous and pancreatogenous diarrheas, with gastrocolic fistula, celiac disease, carcinoma of the stomach, and after subtotal resection of the stomach. If the laboratory diagnosis is correct, if the antianemic preparations are potent and of sufficient dosage, and if the erythrocytic mechanism is not entirely exhausted, then the patient's blood picture will improve even though the disease is hopeless. This observation has been confirmed by satisfactory responses in the blood of patients suffering with carcinoma of the stomach.

The third group includes those anemias with a volume index lower than normal and a color index comparably low. The cells are adequately filled with pigment. They range in size from 72 to 79 cubic microns, have from 22 to 26 micromicrograms of hemoglobin and present a concentration of hemoglobin of 31 to 38 per cent. This type of anemia is the variety usually found in chronic infections including typhoid, in cardiorenal disease, bronchiectasis, in lung abscess, and in septicemia. In this same category are found the anemias of malignancy uncomplicated by hemorrhage, secondary infection, or bone marrow involvement. Sometimes acholuric jaundice exhibits a macrocytosis. Unfortunately we have no direct therapeutic answer for this group. If the cause is evident and if it is treatable, then one should obviously attack the cause. If the laboratory findings place an anemia in this group, it becomes evident that little is to be expected from the usual antianemic measures.

The fourth and last group includes those patients with anemias whose indices are unusually low. The cells measure from 50 to 71 cubic microns, they contain only from 14 to 21 micromicrograms of hemoglobin, and the hemoglobin concentration ranges from 24 per cent to 29 per cent. This group is styled hypochromic microcytic because the cells are poorly saturated with pigment and small in size. Chronic hemorrhage from any source will eventually produce this picture. The same type is noted in a number of anemic states variously known as chlorosis, simple hypochromic and achlorhydric anemias. These varieties at times are only moderately microcytic. During pregnancy one may find a state of hypochromia as the dominant deviation. Myxedema, scurvy, beriberi, pellagra, hookworm infestation, poor nutritional states in infants, gastrointestinal resection and stricture, and the several grave diarrheal states are some of the conditions in which we may find this combination of microcytic hypochromia. This is the type of anemia, lacking acutely in pigment content,

that has responded well in the past, after the exhibition of iron, and that responds even more satisfactorily today because of the increased doses of iron used. Copper in all likelihood contributes considerably to the relief of patients in this group, although no particular attention needs to be paid to the deficiency because sufficient copper will be available in the usual iron preparations and in an adequate diet. Vitamin C and thyroxin are also needed at appropriate times by patients who present this variety of anemia. In this instance, also, the secondary anemia fraction found in liver when combined with iron, will produce gratifying results. The collateral use of large doses of dilute hydrochloric acid will undoubtedly hasten recovery for those who are afflicted with this sort of anemia and who are achlorhydric. The absence of hydrochloric acid and the gastric enzymes is established by the laboratory.

In addition to the establishment of all the foregoing facts and factors through laboratory procedure, it is the laboratory to which the physician must look for assistance in making a decision as to whether or not his patient is the victim of a leucosis, whether he has an intestinal infestation, whether there is an achylia or achlorhydria, what type of diarrhea may be present, whether there is a septicemia or bacteremia, whether there is a granulopenic or thrombopenic state. In doubtful cases the pathologist in the laboratory may illumine a clouded situation by examination of the bone marrow of sternal biopsies, to say nothing of the aid which may come from the roentgenologic laboratory.

It is not to be inferred, however, that laboratory investigation in the anemias precludes all other studies. It is still good practice to obtain thorough histories, and to examine the patient well before outlining logical laboratory investigation. From the many disease entities mentioned in this presentation, it is evident that anemia occurs under many and varied circumstances. Much more than laboratory reports must be had before complete and intelligent diagnoses can be made and before comprehensive and effective therapy can be applied. Exact diagnoses can, after all, here as elsewhere, result only after every bit of evidence is obtained by *all* the means at the command of the physician; and treatment cannot be directed from the laboratory.

It may, at this point, occur to the reader that the approach outlined here is, after all, in accord with usual practice, and that it poses no new doctrine. This may be substantially true. It is hoped, however, that this presentation does clarify certain factors in our present tendencies, and emphasizes certain dangers due to attempts to experiment in several directions at the same time. It is not unusual, for instance, to find all three of the major antianemic attacks being applied at once. This is unscientific and certainly, to say the least, uneconomical. It also makes for difficulty in gauging dosage and in evaluating results. In other forms of therapy, we deprecate shotgun prescription, why not in antianemic therapy? It is not unusual also to find an assumption that the patient has pernicious anemia governing the use of liver or ventriculin therapy. No results are forthcoming and therapeutic skepticism is immediately created. Perhaps a careful analysis of the indices might show a lack of pigment saturation and a need for iron instead of liver, or perhaps the analysis

might reveal anemia requiring other methods. It should be said, in passing, that the almost universal use of liver or other antipernicious anemia preparations for all types of anemia has unfavorably influenced the cost of treatment for that group that specifically needs this.

*In Conclusion.*—While exclusive reliance is not to be placed upon the laboratory in the study of anemic patients and in the control of their treatment, we cannot intelligently forego the measurable directional aid that will be available if certain laboratory studies are called for in intelligent fashion, and then evaluated in the light of clinical judgment and experience. Patients should not be treated uniformly. If we approach *by way of the laboratory, in part at least*, the exceedingly difficult problems presented in the diagnosis and treatment of the anemias, the attack will be direct, the control intelligent and the therapeutic results as good as can be expected.

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## THE EFFECT OF JAUNDICE ON INTRADERMALLY INJECTED SALT SOLUTION\*

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J. M. MORA, M.D., AND F. J. JIRKA, M.D., CHICAGO, ILL.

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SINCE the intracutaneous salt solution test was described by McClure and Aldrich in 1923,<sup>1</sup> it has been studied in a variety of conditions with interesting results. Normally, the wheal produced by injecting 0.2 c.c. of 0.85 per cent aqueous solution of sodium chloride intradermally persists for sixty minutes or more. McClure and Aldrich<sup>2</sup> noted rapid disappearance of the wheal in edema, the disappearance time being roughly in inverse ratio to the degree of edema at the site of injection. Similar reduction of the disappearance time of the intradermally injected salt solution was subsequently noted in acute infections as scarlet fever and diphtheria,<sup>3</sup> lobar pneumonia,<sup>4</sup> toxemias of pregnancy,<sup>5</sup> areas of deficient circulation,<sup>6</sup> serum sickness,<sup>7</sup> and thyrotoxicosis.<sup>8</sup> Prolongation of the disappearance time was found by Thompson<sup>9</sup> in myxedema. White and Irvine Jones<sup>10</sup> have shown that the disappearance time is not affected by the hydrogen ion concentration, the osmotic pressure, or the salt balance of the injected fluid. Neither the blood flow through the injected part nor the immersion of the injected extremity in hot or cold water has any notable effect. The disappearance time is also not influenced by the concentration of the nonprotein nitrogen, cholesterol, or sodium chloride in the blood plasma. It is, however, definitely diminished by injecting minute amounts of adrenalin with the salt solution or by the direct application of cold to the local area of injection.

In the course of some other work, we noted rapid disappearance of the saline wheal in cases of jaundice. Investigating the matter further we studied

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\*From the Department of Surgery, University of Illinois College of Medicine, and the Cook County Hospital (Service of Dr. F. J. Jirka).  
Received for publication, August 28, 1931.

carefully fifteen cases of well-marked jaundice (of hepatic origin). The series included eleven women and four men; the youngest was twenty-nine years of age, the oldest sixty-five years. The nature of the lesion in each case is given in Table I. The test was performed according to the original technic of McClure and Aldrich, the wheals being made on the flexor surface of the right forearm below the cubital fossa, and the end-point determined by palpation.

TABLE I  
TABLE SHOWING COMPLETE PROTOCOL

NAME	AGE	SEX	LESION	DISAPPEARANCE TIME (MINUTES)		ICTERUS INDEX	
1. G. W.	50	F.	Syphilitic cirrhosis of liver	(1) 30 (2) 18 (3) 6		(1) 27 (2) 34 (3) 40	
2. M. G.	65	M.	Carcinoma of head of pancreas	(1) 28 (2) 22 (3) 7		(1) 42 (2) 44 (3) 60	
3. F. L.	53	M.	Carcinoma of common bile duct	(1) 18 (2) 12 (3) 4		(1) 62 (2) 75 (3) 112	
4. M. W.*	54	F.	Cholelithiasis	(1) 32 (4) 70 (2) 32 (5) 64 (3) 48 (6) 80		(1) 48 (4) 11 (2) 42 (5) 7 (3) 24	
5. C. L.*	39	F.	Cholelithiasis	(1) 24 (4) 65 (2) 37 (5) 68 (3) 48 (6) 78		(1) 60 (4) 12 (2) 42 (5) 9 (3) 30	
6. K. A.*	50	F.	Cholelithiasis	(1) 38 (4) 75 (2) 30 (5) 84 (3) 52 (6) 78		(1) 52 (4) 18 (2) 26 (5) 8 (3) 22	
7. I. M.	29	F.	Acute catarrhal jaundice	(1) 46 (4) 58 (2) 34 (5) 80 (3) 22		(1) 64 (3) 22 (2) 90 (4) 10	
8. C. S.	49	F.	Toxic hepatitis (drug poisoning)	(1) 22 (4) 14 (2) 17 (5) 10 (3) 14		(1) 72 (3) 70 (2) 64 (4) 84	
9. A. M.	58	F.	Carcinoma of head of pancreas	(1) 35 (4) 40 (2) 37 (5) 36 (3) 35		(1) 30 (3) 48 (2) 36 (4) 54	
10. A. H.	58	F.	Carcinoma of head of pancreas	(1) 40 (4) 22 (2) 31 (5) 56 (3) 26		(1) 18 (3) 36 (2) 30 (4) 44	
11. O. T.*	52	F.	Cholelithiasis	(1) 38 (4) 40 (2) 30 (5) 56 (3) 24 (6) 70 (7) 72		(1) 30 (4) 30 (2) 36 (5) 18 (3) 44 (6) 12	
12. M. D.*	49	F.	Carcinoma of head of pancreas	(1) 28 (4) 34 (2) 15 (5) 54 (3) 30 (6) 66 (7) 62		(1) 70 (3) 24 (2) 38 (4) 14	
13. M. C.*	45	F.	Cholelithiasis	(1) 34 (3) 65 (2) 45 (4) 68		(1) 38 (2) 18 (3) 10	
14. W. S.	67	M.	Carcinoma of rectum with hepatic metastases	(1) 44 (2) 28 (3) 20		(1) 22 (2) 40 (3) 76	
15. I. M.*	50	M.	Cholelithiasis	(1) 30 (3) 56 (2) 32 (4) 72		(1) 46 (2) 20 (3) 12	

\*The asterisk indicates that some operative procedure was carried out designed to correct the pathologic condition and diminish the jaundice.

The disappearance time of the wheal (in minutes) as given in the table represents the average of many tests performed at intervals, and checked up by determinations of the icterus index. A control series of injections was made in another group of patients with gallbladder and hepatic disease without jaundice. Care was taken to exclude from this study any patient exhibiting fever and cardiac or renal damage.

In fourteen of the fifteen cases studied it was found that as the jaundice increased the disappearance time of the saline wheal decreased, and when the jaundice began to diminish, the disappearance time became prolonged and approached or reached the normal. In only one case (Case 9, Cook County Hospital No. 1,309,634) was there no demonstrable change. (No explanation for this is offered at present but the suggestion is ventured that there may have been irreversible chemical changes in the cells because of the jaundice.)

The exact mechanism of this interesting test is not entirely clear. Guggenheimer and Hirsch<sup>11</sup> offered a mechanical hypothesis to explain the rapid absorption of salt solution by edematous tissues. Cohen<sup>12</sup> and his colleagues believe that the mechanism of reaction is that of an increased affinity of the tissues for the water, based largely on the work of Fischer<sup>13</sup> who postulated that the normal turgor of a tissue depends on the physical state of its colloids and that edema results whenever the water-holding power of its colloids is increased and when a supply of free water is available. It is possible that the profound physicochemical changes occasioned by jaundice injure the cells in such a way as to alter their permeability or their chemical reaction. Belicoff<sup>14</sup> calls attention to the fact that the skin is an active organ, capable of reacting to various stimuli, and able to bring into play all available defensive mechanisms. Nadel's<sup>15</sup> work seems to show that the resorption of intracutaneous physiologic salt solution depends on several factors: the state of the peripheral blood vessels, the vegetative nervous system, elasticity of the skin, temperature, degree of intoxication, age, etc. The chemical changes in the skin may not be without some significance. The shortening of the disappearance time, therefore, cannot be looked upon as entirely due to tissue thirst. Adlersberg and Perutz<sup>16</sup> have even produced some evidence of a centrally acting mechanism.

#### SUMMARY

The intracutaneous salt solution test was studied in fifteen cases of jaundice (hepatic). In fourteen of the fifteen cases there was a definite relationship between the severity of the jaundice and the disappearance time of the saline wheal. With increasing jaundice the disappearance time of the saline wheal decreased; with diminution in the severity of the jaundice the disappearance time of wheal became prolonged and approached or reached the normal.

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## THE DIFFERENTIAL COUNT AT HIGH ALTITUDES\*

R. F. PETERSON, M.D., BUTTE, MONT., AND  
W. G. PETERSON, WARREN, MINN.

IN 1933, Stammers<sup>1</sup> reported a study of the polymorphonuclear-lymphocytic ratio at an altitude of 5,750 feet. This altitude corresponded so closely to that at Butte, Montana, that it was decided to make a corresponding study here. The official altitude of Butte (Lat. 45° 57' N.; Long. 112° 30' W.) is 5,755 feet, although it varies from 5,527 feet to about 6,400 feet.

Stammers found averages of 54.20 per cent polymorphonuclears and 39.72 per cent lymphocytes in his study of blood pictures in 171 healthy young adults. This percentage of polymorphonuclears was 14 per cent below, and that of lymphocytes approximately the same amount above the respective averages of 68.2 per cent polymorphonuclears and 25.8 per cent lymphocytes which he found in twelve textbooks. These averages were presumably given for areas near or at sea level. Almost all the textbooks which we use give percentages slightly lower than 68.2 per cent for the polymorphonuclears, and also give the figures in round numbers, such as "60-70 per cent," "about 65 per cent," and "62-64 per cent." Earhart,<sup>2</sup> quoting Schilling, gives the normal range for polymorphonuclears as 54 to 72 per cent, the average being 67 per cent. Together they average about 65 per cent.

Clark's<sup>3</sup> experiments and survey of the literature show that ultraviolet light stimulates a relative lymphocytosis in both man and animals. People living in relatively higher altitudes would naturally be exposed to more ultraviolet radiation from the sun than those living at lower altitudes, and this fact is given as the most plausible explanation for relative lymphocytosis at these altitudes.

Johannesburg, Africa (Lat. 26° 11'0), where Stammers made his study is much closer to the equator than is Butte. It also receives a greater percentage of sunlight, 73 per cent, compared with 57 per cent for Butte;† Switzerland 40 per cent; and London 29 per cent. The logical conclusion from these facts is that it receives even more ultraviolet radiation than Butte.

The following study was made on young healthy adults whose ages averaged 24.3 years, and most of whom had lived at this altitude all their lives, though a few had been here for shorter periods. They were nearly all prospective blood donors or students, and all were questioned about illnesses, especially colds. As said before, the study was primarily undertaken to determine the polymorphonuclear-lymphocytic ratio, but a complete blood count was done

\*From the Murray Clinic, Butte, Mont.

Received for publication, September 14, 1934.

†Figure for Helena, Mont., north of Butte.

on all subjects to exclude any possibility of deviation due to unexpected variation in hemoglobin, red or white cell counts. Only one subject was excluded and that was a woman who showed a marked secondary anemia, though it was later seen that her polymorphonuclear ratio was practically the same as the average of the whole group. Thirteen showed unexplained white counts over ten thousand, from 10,500 to 15,100, but the average polymorphonuclear ratio of these was only about 2 per cent higher than the total of the whole group.

Alvarez<sup>4</sup> has found that at sea level a barometric low produces a temporary leucocytosis. The barometer at Butte usually hovers around 618 mm. of mercury, with variations to approximate limits of 600 mm. and 625 mm. The deduction from these facts could be made that the low pressure increased the total number of leucocytes, but so-called normal variations of the total white counts are probably higher than usually given. Experiments at the Washington Square College of New York University<sup>5</sup> revealed white counts ranging from 4,700 to 11,500 in persons at rest, and that mild activity produced no constant effect on the count. In our study, the low and high counts were 4,050 and 15,100, respectively, the average on all subjects being 8,260.

The hemoglobin readings were done with the Dare, and were read and reread by two persons familiar with that instrument. The red and white counts were thoroughly mixed by automatic shaker and by hand before being counted. Smears were stained with Wright's stain and 300 cells were counted from all portions of the slide unless the distribution was uneven, when more were counted.

One hundred subjects were studied, 25 women and 75 men. The following tables of hemoglobins, red and white counts are not from a large enough series to permit of definite conclusions but are of interest in addition to the main study.

TABLE I

	HEMOGLOBIN (DARE)	ERYTHROCYTES	LEUCOCYTES
Average of all (100)	85.88%	5,037,600	8,260
Average of men (75)	86.78%	5,172,000	8,350
Average of women (25)	83.16%	4,636,000	7,980

TABLE II

THE RESULTS OF THE DIFFERENTIAL COUNTS

CELLS	RANGE	MEAN
Polymorphonuclears	35.5-72.5	54.36
Lymphocytes	19.0-53.0	36.26
Monocytes	2.5-15.0	6.33
Eosinophiles	0.0- 6.5	2.00
Basophiles	0.0- 2.0	0.645

In comparison to this, Stammers found a mean of 54.20 per cent for polymorphonuclears.

Sachs,<sup>6</sup> working in Transvaal at an altitude of 4,392 feet above sea level, found a mean of 54.70 per cent in 81 individuals, so that these three different results are within 0.5 point of each other.

Ruppanner<sup>3</sup> examined the bloods of healthy adults at altitudes of 1,750 meters (5,741 feet) and 2,250 meters (7,382 feet) above sea level in the Swiss mountains with the following results:

1. For eight native men, altitude 1,750 m. (5,741 feet) the average leucocytic formula was as follows:

Total leucocytes 5,775	
Neutrophile	49.85%
Lymphocyte	38.25%
Large mononuclears and transitional forms	8.85%
Eosinophile	2.75%
Mast cells	0.3 %

2. In twelve native women, altitude 1,750 m. (5,741 feet)

Total leucocytes 7,250	
Neutrophile	59.00%
Lymphocyte	31.4 %
Large mononuclears and transitional forms	7.35%
Eosinophile	1.85%
Mast cells	0.4 %

3. In thirty-nine men visiting for a period of from several weeks to a year, altitude 1,750 m. (5,741 feet)

Total leucocytes 6,740	
Neutrophile	58.75%
Lymphocyte	20.2 %
Large mononuclears and transitional forms	8.6 %
Eosinophile	2.95%
Mast cells	0.5 %

4. Visiting women, altitude 1,750 m. (5,741 feet)

Total leucocytes 7,680	
Neutrophile	60.0 %
Lymphocyte	30.75%
Large mononuclears and transitional forms	7.0 %
Eosinophile	1.6 %
Mast cells	0.3 %

5. Twelve men, altitude 2,250 m. (7,382 feet)

Total leucocytes 7,670	
Neutrophile	50.2 %
Lymphocyte	36.6 %
Large mononuclears and transitional forms	9.0 %
Eosinophile	4.0 %
Mast cells	0.2 %

After examining a group of healthy subjects immediately after and for some time after their arrival in the mountains, Ruppanner states that the evidence indicated that the normal leucocytic formula at high altitudes shows a normal or slightly lower total number of leucocytes, neutropenia, lymphocytosis, and a slight degree of mononucleosis.

Hartman<sup>4</sup> studied the bloods of members of the German expedition to the Himalayas in 1931 and gives the results as shown in Table III.

He observed signs of regeneration more marked in the red cells at high altitudes than at low altitudes.

TABLE III

TYPES OF CELLS	ELEVATION IN METERS								
	0	2800 (9184) FT.	4400 (14,432) FT.	5600 (18,368) FT.	6300 (20,664) FT.	6600 (21,648) FT.	7200 (23,616) FT.	7600 (24,928) FT.	600 (1968) FT.
Eosinophile	3%	5%	2%	3%	3%	3%	3%	2%	3%
Rod-shaped nucleus	1	2	1	1	1	1	3	1	1
Segmented nucleus	64	44	50	54	56	58	61	63	52
Lymphocytes	29	45	42	39	37	32	27	29	40
Large mono-nuclears	3	4	5	3	3	6	6	5	4

An unrecalled reference stated that the eosinophiles increased in percentage toward summer as the sun's rays became stronger. It happened that this work was done in the winter of 1933-1934 and the spring of 1934. The two seasons were arbitrarily divided in March and the first 57 taken in the winter showed an average of 1.87 per cent eosinophiles; the last 42 taken in the spring showed an average of 2.21 per cent eosinophiles. These figures would tend to bear out the findings of the writer whom we cannot recall.

## SUMMARY

1. Our findings agree with those of other writers that a relative lymphocytosis occurs at high altitudes.

2. This means a decrease of from 10 to 14 per cent in the polymorphonuclears in contrast to what might be expected at sea level, which fact must be taken into consideration when making a diagnosis or prognosis.

3. A slight increase (0.34 per cent) in eosinophiles was found in the counts taken during the spring months as compared to those taken during the winter months.

4. Thirteen per cent of the group of 100 healthy young persons are found to have a total leucocytic count over the usually accepted upper normal limit of 10,000.

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# ARE BRUCELLA ABORTUS AGGLUTININS IN THE BLOOD STREAM PRODUCED BY ACTIVE OR PASSIVE IMMUNIZATION?\*

C. EMELIA PETERSON, A.B., M.A., SAN FRANCISCO, CALIF.

## INTRODUCTION AND HISTORICAL

THIS study was undertaken in an attempt to determine whether the presence of *Brucella abortus* agglutinins in blood serum indicates active or passive immunity. When this work was started there was a question as to whether specific abortus agglutinins in human blood were of sufficient diagnostic value to determine active abortus infection when cultural findings from the blood and urine are negative. Although it was later found that *Brucella abortus* does occur in the blood stream of man, the above question still remained of academic interest, as its interpretation is of significance in cases where a high agglutination titer is obtained when the blood and urine cultures are negative.

Specific agglutinins for *Brucella abortus* in the blood stream of human beings have been reported by various investigators who were unable to determine whether their results indicated active or passive immunity. Larson and Sedgwick,<sup>1, 2</sup> after examining the blood sera of 425 children consuming market milk, found 17 per cent to contain antibodies against *Brucella abortus* by the agglutination and complement fixation tests. In two other groups 40 per cent and 48 per cent of the serums, respectively, were positive for *Brucella abortus*. As they had previously succeeded in infecting guinea pigs by feeding market milk known to contain *Brucella abortus*, they did not believe passive immunity so highly probable but were of the opinion that active immunity may have arisen as a result of infection. Children fed milk from a clean herd did not develop antibodies for *Brucella abortus*. Cooledge<sup>3</sup> fed raw milk of high antibody content to human subjects and concluded that antibodies in the blood stream of human beings are the result of passive immunity due to the absorption of antibodies from the milk and not to infection. The fact that the individuals were apparently healthy at all times during the feeding experiments indicated to him that there was no active immunity. Wilson and Nutt<sup>4</sup> stated that antibodies were present in a certain proportion of milk-consuming children and in a still lower proportion of adults. They were doubtful as to whether this was the result of infection or absorption of antibodies present in the milk. Carpenter and Boak<sup>5</sup> fed heated milk containing *Brucella abortus* and their respective agglutinins in high titer to two groups of male students. They were unable to demonstrate agglutinins in their blood

\*From the Department of Public Health and Preventive Medicine, Stanford University School of Medicine.

Received for publication, September 18, 1934.

serums and concluded that *Brucella abortus* agglutinins in human blood serum are the result of active infection. Carpenter and King<sup>3</sup> state in their "Symposium on Undulant Fever" that "the presence of specific abortus agglutinins is evidently a result of an active infection instead of a passive absorption of such antibodies from drinking milk containing them." They contend that undoubtedly some of the agglutinins from the milk serum are absorbed, but because of the great dilution factor due to the amount of blood in the tissues, they cannot be demonstrated.

#### EXPERIMENTAL

A series of experiments was undertaken in which groups of guinea pigs were fed living *Brucella abortus* cultures and *Brucella abortus* agglutinins. As recent reports of successful oral vaccination suggested that active immunity might follow the ingestion of dead bacteria, a series of tests using killed *Brucella abortus* was also included.

#### PROCEDURE

Healthy normal guinea pigs were placed in clean metal cages in groups of six. Each cage was supplied with from 200 c.c. to 350 c.c. of pasteurized milk daily, this not only serving as a substitute for water but also as a convenient means of administering the antigens and serum in desired amounts. Two strains of *Brucella abortus*, C M-90 isolated from certified milk and C6-3 cultured from the spleen of a guinea pig inoculated with milk from a cow infected with *Brucella abortus*, were employed in feeding. On finding these to have become unsatisfactory, strains H-1751 and H-1767 isolated from calf fetuses, were obtained from Professor Hart of the University of California. Suspensions of living bacteria were made by washing up forty-eight-hour Blake bottle cultures in 50 c.c. of sterile physiologic salt solution. Killed antigens were similarly prepared by heating for one hour at 60° C. or by adding 0.18 per cent formalin, sterility tests being carefully checked before use. Uniform dosages were insured by standardization in Hopkins vaccine tubes, 6.25 c.c. of a 1 per cent suspension or its equivalent constituting the daily dosages (approximately 10 billion bacteria).

Specific agglutinins were obtained by inoculating goats subcutaneously with 5 c.c. of killed *Brucella abortus* at weekly intervals. Titers, checked once a month, ranged from 1-5,120 to 1-10,240, sufficient serum being added to the milk to give the desired titer.

After each guinea pig was weighed and found to be negative for *Brucella abortus* agglutinins, feeding was commenced. Weights and titers were recorded once a month during the period of feeding. If the agglutination tests were positive in an appreciable titer, the animals were sacrificed and examined for typical lesions of *Brucella abortus* infection. Spleens showing enlargement or suspicious lesions were cultured on plain agar slants and incubated in 10 per cent carbon dioxide. Growth of organisms morphologically resembling *Brucella abortus* was serologically checked by the agglutination test. Feeding was continued for several months if the animals had developed no titers.

## RESULTS

*Experiment I.*—Two series of guinea pigs were run concurrently using two strains of *Brucella abortus*, C6-3 and CM-90.

*Series (1):* Two sets of six guinea pigs each were placed in separate cages, one set being fed 6.25 c.c. of a 1 per cent suspension of living *Brucella abortus* C6-3 in 350 c.c. of milk, and the other 350 c.c. of milk only.

Daily feeding was discontinued thirteen days later upon finding this strain of *Brucella abortus* to have become avirulent and overgrown with gram-positive sporulating rods. A fresh antigen was prepared from strain H-1751. The animals were rechecked for abortus agglutinins and on being found negative, feeding was resumed again in five weeks.

One month later titrations were made. The animals fed living culture showed titers ranging from I-320 to I-1280. Autopsies revealed swollen, mottled spleens ranging from one and one-half to four times normal in size. Cultures showed pure growth of *Brucella abortus*. The controls showed no agglutinins at this time, one having died from an undetermined cause. Three and one-half months later, three controls gave titers of I-10 and cultures of the spleens of two of these showed growth of *Brucella abortus*.\* Cultures made from the spleen of the remaining pig and the two others which developed no agglutinins, were negative after prolonged incubation, although one showed growth of a streptococcus.

*Series (2A):* Two sets of six guinea pigs each were fed 350 c.c. of milk containing 6.25 c.c. of a 1 per cent suspension of living *Brucella abortus* CM-90 and 350 c.c. of milk, respectively. After thirteen days, feeding was discontinued as this strain of *Brucella abortus* grew very sparsely making it difficult to prepare sufficiently large amounts of antigen.

Five weeks later, before feeding was to be resumed with a new antigen, titrations were made. The living culture series was found to agglutinate *Brucella abortus* completely in a dilution of I-160. A subsequent titration sixteen days later showed titers ranging from I-640 to I-10,240. Autopsy showed spleens in five guinea pigs which were mottled and enlarged from one and one-half to three times. All gave pure cultures of *Brucella abortus*. The controls had developed no agglutinins. Spleens of all controls were normal in appearance and cultures were negative for *Brucella abortus*.

*Series (2B):* The experiment was resumed by feeding six guinea pigs 6.25 c.c. of a 1 per cent suspension of living *Brucella abortus* H-1767 in 350 c.c. of milk, and another six animals 350 c.c. of milk only.

In twenty-one days, the guinea pigs fed living organisms had lost weight and had developed titers ranging from I-40 to I-80. Feeding was continued and five weeks later titers ranged from I-1,280 to I-10,240. At autopsy, one spleen was normal in size, the remaining five being enlarged two to four times. All were characteristically mottled and *Brucella abortus* was recovered in pure culture in each case. Of the control series, five were still living at

\*Investigation determined that in daily cleaning of the cages, the feeding pans of the control series had been contaminated with small doses of *Brucella abortus*, thus accounting for the discrepancies. This was carefully controlled in all later experiments.

three and one half months, one having died previously from an unknown cause. Three gave suspicious agglutination tests in I-10 and two were negative. Autopsy findings and cultures of all were negative for *Brucella abortus*, one culture, however, showing a streptococcus contamination.

It is evident from these experiments that living *Brucella abortus* administered orally to guinea pigs results in active infection. Agglutinins in relatively high titers are developed and autopsy findings reveal typically mottled and usually enlarged spleens from which *Brucella abortus* can be successfully isolated.

*Experiment II.*—Twelve guinea pigs were separated into two cases of six animals each. The animals in one cage were fed 350 c.c. of milk containing *Brucella abortus* agglutinins in a titer of I-80 which is higher than any titer observed in raw milk in this laboratory, those in the other cage receiving milk only. After daily feeding over a period of seven months, no agglutinins were detected in the blood of the test series, the controls likewise being negative.

A suggestion was made by Professor Hart of the University of California that a I-80 dilution of *Brucella abortus* agglutinins might not be sufficient to induce passive immunity in guinea pigs to a degree high enough to be detected by the agglutination test. By verbal communication, he informed us that he had seen cow's milk with titers considerably higher than I-80.

Fourteen young weaned guinea pigs weighing from 150 to 200 gm. were segregated into two cages of seven animals each. Those in one cage were fed 200 c.c. of pasteurized milk and sufficient goat serum to make an agglutinin titer of I-640 which is eight times as concentrated as in the previous experiment. Those in the other cage received milk only. One of the test pigs died after two months from an unknown cause. Two control animals died within several months of streptococcus infections and one died after the first test bleeding. Titrations made at one- or two-month intervals showed no agglutinins for *Brucella abortus* in the test or control series after daily feeding over a period of five months.

These findings indicate that the daily administration of *Brucella abortus* agglutinins to guinea pigs per os over a period of several months does not result in absorption of agglutinins to such an extent as to be detected by the agglutination test.

*Experiment III.*—Comparatively recent studies in the field of immunology have resulted in reports by various investigators on the possibility of successful vaccination by the oral route. This subject has been reviewed by Enlows<sup>7</sup> and suffice it to mention that Hoffstadt and Thompson<sup>8</sup> have recently produced agglutinins in the blood serums of human subjects by oral inoculation of triple typhoid vaccine. They found no delay in the appearance of agglutinins over that of the subcutaneous method. Bile seemed to be an aid in the production of these antibodies.

Two antigens, one killed with heat and the other with formalin, were prepared with each of the following organisms: *Brucella abortus* H-1751, *B. typhosus* Rawlings, and *B. dysenteriae* Shiga #204 (American Type Collection). Sterility tests were carefully checked before use and all cages and



feeding pans were thoroughly cleaned and disinfected. After having been checked for the absence of agglutinins, six groups of six guinea pigs each were administered the above antigens in 300 c.c. milk, respectively. A seventh group of six animals, receiving milk only, served as a control.

After daily feeding for seven months, no agglutinins had been stimulated by either antigen of the organisms tested. The controls were also negative.

The effect of bile on the stimulation of agglutinins in guinea pigs following oral administration of these bacteria was studied next. On being found free from agglutinins, each animal in cages of six, was fed 2.5 c.c. of bile in licorice powder. After being deprived of food and water for twenty-four hours, they were fed formalinized antigens of *Brucella abortus*, *B. typhosus* Rawlings, and *B. dysenteriae* Shiga in 250 c.c. of milk, a control set receiving milk only.

Daily feeding was continued for one month and at the end of this period, titrations failed to detect the presence of agglutinins for *Brucella abortus*, *B. dysenteriae* Shiga, and *B. typhosus*.

These experiments indicate that in guinea pigs the administration by mouth of large doses of certain killed bacteria daily even over a period of seven months does not stimulate the production of antibodies which can be detected by the agglutination test. Preparation of the intestinal mucosa with bile also failed to stimulate the production of these antibodies.

#### DISCUSSION

These results show that in guinea pigs agglutinins arise following an active infection with *Brucella abortus*. Dead bacteria administered by mouth in large doses do not stimulate the production of agglutinins in guinea pigs even though the intestine is previously prepared with bile. The administration of abortus agglutinins by mouth eight times as concentrated as usually found in infected milk does not result in absorption of antibodies to a degree that can be detected by serologic tests. The development of agglutinins for *Brucella abortus* in the blood of guinea pigs only after the administration of living bacteria indicates that agglutinins are the result of an active immunity following infection.

By analogy, these experiments indicate that *Brucella abortus* agglutinins in the blood stream of human beings are undoubtedly the result of infection as first expressed by Larson and Sedgwick.<sup>1, 2</sup> The feeding of large amounts of high titered serum daily to guinea pigs over a period of several months with no appearance of agglutinins would seem to indicate that agglutinins in human blood also have not been absorbed through the intestinal wall from milk consumed and are, therefore, not due to passive immunity as expressed by Cooledge.<sup>3</sup> The stimulation of active immunity by dead bacteria which might be present in pasteurized milk seems unlikely, as the number of bacteria present in milk for human consumption certainly would not compare with the excessive doses of bacteria used in these experiments. Therefore, *Brucella abortus* agglutinins in human blood must indicate infection and active immunity, and their presence would suggest present or past infection even though urine and blood cultures are negative.

## CONCLUSIONS

1. Daily feeding of specific abortus agglutinins over a period of seven months in the concentration usually found in milk did not result in the detection of agglutinins in the blood of guinea pigs. Similar feeding over a period of five months with abortus agglutinins in a concentration eight times as great failed to show the presence of these antibodies in the blood of guinea pigs.

2. Daily feeding of killed *Brucella abortus* cultures over a period of seven months did not result in the formation of agglutinins in the blood of guinea pigs. Results were also negative even though the intestinal mucosa was previously prepared with bile. Control tests with *B. typhosus* and *B. dysenteriae* Shiga showed no development of agglutinins for these organisms.

3. Daily feeding of living *Brucella abortus* cultures to guinea pigs produced an active infection which was confirmed by agglutination tests and by recovery of *Brucella abortus* in pure culture from the spleens at autopsy.

4. Our results indicate that in guinea pigs, specific abortus agglutinins are not due to passive immunity or reaction to killed abortus antigen, but are due to active infection with *Brucella abortus*.

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## EOSINOPHILIA IN SYPHILIS\*

### A COMPARATIVE STUDY OF THE DIFFERENTIAL LEUCOCYTE COUNTS IN 100 POSITIVE AND 100 NEGATIVE BLOOD WASSERMANN CASES

RALPH H. SPANGLER, M.D., PHILADELPHIA, PA.

THE practice of making routine differential leucocyte counts on all allergic patients and also in a majority of other new patients examined, and the finding of an eosinophilia (5 to 10 per cent and at times higher) in an occasional patient having no allergic clinical manifestations, a negative history of syphilis, and showing no syphilitic symptoms, but whose blood Wassermann test subsequently proved positive, has interested me for a number of years.

A recent connection with the Medical Service of the Philadelphia County Prison, with its vast amount of clinical material among a yearly turnover of more than 30,000 men, offered the opportunity, through the courtesy of Dr. C. Y. White, director of Philadelphia Municipal Laboratories who had the Wassermann tests made, to make a comparative study of the differential leucocyte counts in 100 men having positive and 100 men with negative blood Wassermann reactions at the Clinical Laboratory in Moyamensing Prison.

Physiologists have not given us much positive information as to the normal function of the blood leucocytes. Numerous hypotheses have been suggested following many investigations as to their function in health and under various pathologic conditions, but the significance of the altered proportional relation of the cells in the differential leucocyte count in connection with various biologic and immunologic reactions is far from being definitely understood.

With our increased knowledge the last few years of the part played by the reticulo-endothelial system in the origin of blood cells, and with more exact evidence on their biologic development we should be able by intensive and careful clinical observation to interpret more definitely the significance of the percentage range of the leucocytes in the differential count. Indeed, the differential leucocyte count seems destined to furnish some of the most important information of all the studies being made of the formed elements of the blood. The interpretation and evaluation of a study of the proportional percentage of the cells in the differential leucocyte count under various physiologic, immunologic and therapeutic conditions is undoubtedly becoming of more aid to the clinician, and it is with this fact in mind that the present report is submitted.

\*Read at the Twelfth Annual Meeting of the Association for the Study of Allergy, Cleveland, Ohio, June 11 and 12, 1934.

Received for publication, August 6, 1934.

## BLOOD FINDINGS IN SYPHILIS

The literature on the whole is rather indefinite when one reviews the reported data on "Blood Findings in Syphilis." Certainly no typical or specific morphologic blood picture in syphilis is recognized, or has been established. Piney<sup>1</sup> says that the relation of syphilitic infection to "blood diseases" has often been discussed, but with very discordant results, and states that the whole subject is extremely obscure. In connection with any blood picture in a patient who has a positive Wassermann reaction, it is important of course to eliminate other diseases. When syphilis is associated with any disease it is uncertain, to say the least, that syphilis is alone the causative factor in the altered blood picture, and it is especially necessary to rule out diseases commonly recognized as allergic in positive Wassermann cases whose differential leucocyte counts show an eosinophilia.

## SYPHILIS AND THE ERYTHROCYTES

A rather extensive literature is available dealing with the effect of syphilis on the red blood cells, hemoglobin, color index, etc. In 1928 Clyde L. Cummer<sup>2</sup> of Cleveland reported a very comprehensive review of the literature on "Anemia and Other Blood Changes in Syphilis," and recorded his own findings in 40 consecutive cases of early (primary and secondary) syphilis. He especially investigated the anemia of syphilis and summing up from his own experience and from reports in the literature he concludes: "that there is no final proof that syphilis may produce an anemia of the pernicious type; that syphilis may merely be associated with anemia and not be its actual cause; that anemia may appear at any stage of the disease and is usually of a secondary type; that in later stages of syphilis anemia may accompany visceral changes, and may be a feature of syphilitic cachexia; and that the anemia of late syphilis may be accompanied by splenomegalia, the clinical picture of which may bear a close resemblance to Banti's disease."

## THE LEUCOCYTES IN SYPHILIS

From reports at hand in the literature the leucocytes in syphilitic infection seem not to have received nearly as comprehensive investigation as have the red cells. Piney<sup>1</sup> says "the number of leucocytes are very different in different cases." He quotes Kyrle<sup>3</sup> (investigation of 100 cases) who found that "there are syphilitics with a normal number of leucocytes, those with a slight leucocytosis and others with a well-marked leucocytosis in about equal proportions." He reports "no relationship between the severity of the infection nor the extent of cutaneous manifestations, and the leucocytic blood picture." He states that "cases with a definite leucocytosis usually show an immediate fall to about normal as a result of treatment."

Pepper and Farley<sup>4</sup> report that "an increase of white cells up to 12,000, and even 20,000 is not unusual in secondary syphilis," and say "that this rise is always due to an actual lymphocytosis; the lymphocytes forming 50 per cent or even over 60 per cent of the increased total count." Stokes<sup>5</sup> states: "The combination of moderate secondary anemia with lymphocytosis in women always deserves a Wassermann test and a careful search for clinical signs."

In Cummer's<sup>2</sup> review of the literature (1928) he states that "in certain cases of syphilis with enlarged liver and spleen the degree of leucocytosis, of the large lymphatic type, is such that leucemia may be suspected," but concludes "that though syphilis may produce the blood changes of leucemia in certain cases, the picture is a simulated one as shown by necropsy observations." A lymphocytic leucocytosis in congenital syphilis is reported by many clinical observers.

Cummer further discussing the effect of syphilis on the leucocyte count quotes Hazen<sup>6</sup> who found that in untreated cases of syphilis there was a slight leucocytosis; that severe cases showed a higher total count and a higher relative and actual neutrophilia than do the milder cases. He felt that patients showing a high lymphocyte and low neutrophile count responded better to treatment than those showing the reverse lymphocyte-neutrophile picture. Cummer also refers to Eason's<sup>7</sup> report of one patient with secondary syphilis in whose blood was found "a fair number of eosinophilic and neutrophilic myelocytes." Zanca<sup>8</sup> under the pseudoleucemic form of congenital syphilitic anemia refers to an "eosinophilic type" which he says is rare, but is accompanied by a great increase of eosinophiles.

In the six differential leucocyte counts recorded in Cummer's series of 40 positive Wassermann cases, one patient showed a relative lymphocytosis, and another patient, complicated with an atrophic arthritis, had a 7 per cent eosinophilia in each of two counts.

I have found no report recording a series of differential leucocyte counts in syphilis, and the findings in the present series of 100 cases appear to be unique so far as it has been possible to correlate them with the available reports furnished by the literature.

#### METHOD OF INVESTIGATION

Differential leucocyte counts were made on 21 patients with positive Wassermanns who showed evidence of *primary lesions*, and on 9 patients who had *secondary symptoms* (skin manifestations). Then coincident with routine taking of blood for Wassermann tests, in prisoners assigned to certain work, blood smears were made until we had accumulated 70 additional differential counts (making a total of 100) on men whose blood Wassermann tests were positive and 100 differential counts on men whose blood Wassermann tests were negative. Among the 70 men with positive Wassermanns who presented no primary or secondary lesions, 23 admitted, after we had received the positive report on their blood, a syphilitic history, and that they had had previous antisyphilitic intravenous injections. Forty-seven, however, of the 70 with positive reactions were typical asymptomatic cases. They had no signs or symptoms of syphilis, denied a previous syphilitic history and did not know that their blood was positive to the Wassermann reaction. In the series of men giving positive reactions no men with a history of diseases commonly recognized as allergic were included, although we found three cases of asthma and four of urticaria among men with positive Wassermann reactions. One man having a positive reaction was also found but not included in the positive series, who had been receiving a bismuth

preparation intramuscularly and had developed a marked exfoliative dermatitis. In this patient total white counts of 26,400, 12,000, and 19,600 were recorded at weekly intervals with the differential counts showing respectively 37, 35, and 38 per cent of eosinophile cells. There was no relative or absolute lymphocytosis present in this case, the leucocytosis being of the polymorphonuclear neutrophilic type. The stools were negative for ova and parasites.

While no cases of allergy were included in accumulating the differential counts in the series of 100 men showing negative blood Wassermann reactions, as a group they cannot all be considered normal subjects, since a few had a cardiovascular or a gastrointestinal disturbance and a number presented various neurotic manifestations for which in most instances there was some physical basis. Among differential counts made on men with negative Wassermann reaction but not included in the tabulated series were 2 cases of asthma, 2 of urticaria, 1 of psoriasis, and 4 of generalized pruritus. Among the men with negative Wassermann reactions was also one man who had eosinophile counts of 20, 25, and 18 per cent, respectively, on different days over an interval of a month. Examination of his stools revealed the presence of *Trichuris trichiura*. The total white counts in this case were 13,600, 15,200, and 10,200, the leucocytosis being of the polymorphonuclear type.

It is realized that it would have made the investigation of more value to have included a total white count in each case and also to have counted 200 to 400 cells instead of only 100 in each differential count, but the circumstances under which the work was done made such a procedure impossible. In 14 of the men having positive reactions we were able to make total white counts which ranged from 5,200 to 12,000 but they are not considered in the analysis. We were, however, able to make eight differential counts on separate days, on each of six men with strongly positive Wassermann reactions and detailed hemograms of this smaller group are appended.

The smears for differential counts were stained with Wright's stain, and in order to eliminate the personal equation in the technical counting of the blood all counts were made by Miss Elizabeth Monaghan, a Kolmer trained technician.

#### THE NORMAL PERCENTAGE RANGE OF LEUCOCYTES IN THE DIFFERENTIAL COUNT

As a basis of comparison and to interpret intelligently the findings in the present investigation, it is necessary briefly to refer to the normal range of the leucocytes in the differential count. Hematologists and physiologists are not agreed in stating what may be considered as the normal percentage range of the granular leucocytes—polymorphonuclear neutrophils, eosinophiles, and basophiles. There is rather general agreement as to the normal range of the lymphocytes and also of the large hyaline monocytes (which include the type of cell formerly designated transitional).

Piney<sup>1</sup> states that in health the polymorphonuclears are about 60 per cent, the eosinophiles 3 or 4 per cent, the basophiles a fraction of 1 per cent, the lymphocytes 20 to 25 per cent, and the monocytes 4 to 8 per cent. Clough<sup>2</sup> says the polymorphonuclears constitute 60 to 65 per cent of the leucocytes in normal blood; eosinophiles 0.5 to 3 per cent; basophiles 0.5 per cent or less;

lymphocytes 20 to 25 per cent; monocytes 6 to 8 per cent. Pepper and Farley<sup>1</sup> say in adult human beings, the polymorphonuclears make up from 55 to 75 per cent of the total white blood cells; eosinophiles 1 to 3 per cent; basophiles 0.5 per cent; lymphocytes 20 to 30 per cent; monocytes 5 to 10 per cent.

Howell's<sup>10</sup> normal range of the various types of white blood cells is wide enough to include the normal limits of most hematologists, and in analyzing and comparing the findings in the present series of differential leucocyte counts in 100 adult males with positive and 100 with negative Wassermann reactions the following normal range as given by Howell has been used: Polymorphonuclears, 60 to 75 per cent; small lymphocytes, 20 to 25 per cent; large lymphocytes, 1 per cent or less; eosinophiles, 4 per cent or under; basophiles, 1 per cent or less; transitionals or hyaline monocytes, 2 to 10 per cent.

There is general agreement that over 4 per cent of eosinophiles in the white blood count shall be regarded as an *eosinophilia*. Hematologists also generally agree there is no clinical advantage in distinguishing between the large and small lymphocytes, and that the normal range of the combined lymphocytes is 20 to 25 per cent. In the present study, therefore, when the combined small and large lymphocytes total over 25 per cent a *lymphocythemia* is regarded as being present; when the polymorphonuclears are above 75 per cent a *neutrophilia* is considered present; and since the basophiles and large monocytes occurred proportionally in so small a percentage in this series and fell well within the recognized normal range they have not been considered.

#### HEMATOLOGIC FINDINGS IN PRESENT STUDY

For the purpose of hematologic analysis and clinical interpretation the differential leucocyte counts in the 100 men with positive and 100 with negative Wassermann reactions in the present study are presented in the following summarized form:

In the series of 100 men with positive blood Wassermann reactions the differential leucocyte counts show:

40% with an eosinophilia	Eosinophiles,	5 to 10%
74% with a lymphocythemia	Combined lymphs,	26 to 50%
10% with a neutrophilia	Polynuclears,	76 to 89%

In the series of 100 men with negative blood Wassermann reactions the differential leucocyte counts show:

4% with an eosinophilia	Eosinophiles,	5 to 6%
68% with a lymphocythemia	Combined lymphs,	25 to 53%
16% with a neutrophilia	Polynuclears,	76 to 87%

When the "percentage range" of the cells in the differential leucocyte counts of the 100 men with positive and the 100 giving negative Wassermann reactions are compared with Howell's "normal range," as given above, several definite and interesting deductions appear. The most striking, however, is the finding that 40 of the 100 men with positive Wassermann reactions show the

presence of an *eosinophilia*, i.e., the eosinophile cells were above 4 per cent, Howell's upper limit of the normal range. Two men had 10 per cent, two 9 per cent, three 8 per cent, nine 7 per cent, eleven 6 per cent, and thirteen 5 per cent of eosinophiles in the differential counts. In only four of the men having positive reactions no eosinophiles were found in counting 100 cells per slide. In the series of 100 men with negative Wassermann reactions, however, only four showed the presence of a moderate eosinophilia. One man had 6 per cent and three had 5 per cent of eosinophile cells in the differential count, while in twenty of the men giving negative Wassermann reactions no eosinophiles were found in counting 100 cells per slide.

The finding of a lymphocytic increase above the normal range in 74 per cent of the positive cases, and of a polymorphonuclear increase above the normal range in only 10 per cent of the positive cases confirm previous reports recorded in the literature that a lymphocytosis is a characteristic finding of syphilitic infection.

#### CLINICAL ANALYSIS

For the consideration of a more detailed clinical analysis and comparison, the differential leucocyte counts in the 100 men with positive Wassermann reactions are separated into four clinical groups which summarized reveal the following:

*Group I Includes 21 Cases With Initial Lesions:*

Nine (42.85%) show an eosinophilia	Eosins, 5 to 10%.
Thirteen (61.90%) show a lymphocythemia	Lymphs, 26 to 48%.
Four (19.04%) show a neutrophilia	Polys, 76 to 85%.

*Group II Includes 9 Cases With Secondary (Skin) Manifestations:*

Four (44.44%) show an eosinophilia	Eosins, 5 to 6%.
Seven (77.77%) show a lymphocythemia	Lymphs, 26 to 45%.
None show a neutrophilia	Polys under 75%.

*Group III Includes 47 Cases Who Never Received Treatment and Who Did Not Know That Their Blood Wassermann Reaction Was Positive:*

Twenty-one (44.68%) show an eosinophilia	Eosins, 5 to 9%.
Thirty-five (74.44%) show a lymphocythemia	Lymphs, 26 to 50%.
Three (6.38%) show a neutrophilia	Polys, 76 to 83%.

*Group IV Includes 23 Cases Who Had Received Previous Intravenous Treatment:*

Six (26.08%) show an eosinophilia	Eosins, 5 to 7%.
Nineteen (82.60%) show a lymphocythemia	Lymphs, 26 to 40%.
Three (13.04%) show a neutrophilia	Polys, 76 to 89%.

#### DISCUSSION OF CLINICAL GROUP FINDINGS

*The Eosinophilic Granulocytes.*—In the entire series of 100 men with positive Wassermann reactions (including the 77 in various stages of syphilitic infection and the 23 who had received chemotherapeutic treatment) 40 per cent showed the presence of a blood eosinophilia. Upon closer analysis in the clinical group classification it is of interest to note in Group 1 (men with initial lesions) and in Group 2 (men with skin manifestations) that the number of men showing an eosinophilia varied but slightly, being 42.85 per cent and 44.44 per cent, respectively.



It is more interesting, however, to find in Group 3 (men who showed no symptoms of syphilis, who gave a negative syphilitic history, and who did not know their blood Wassermann was positive) that the percentage of cases showing an eosinophilia is even a fractional percentage higher, 44.68 per cent, than in patients with primary and secondary lesions (Groups 1 and 2). Then in Group 4 (men who had received intravenous therapy) we find that the number of men showing an eosinophilia drops to 26.08 per cent, slightly more than half as high as the untreated men in various stages of the disease (Groups 1, 2, and 3).

*The Lymphocytes.*—The combined small and large lymphocytes in the entire series of 100 men with positive Wassermann reactions were above Howell's normal range (20 to 25 per cent) in 74 per cent of the cases. In Group 1, the men showing initial lesions, 61.90 per cent showed a lymphocythemia; in Group 2, men with secondary (skin) manifestations, the number of men showing an increase of lymphocytes above the normal range was 77.77 per cent; in Group 3, men who had no symptoms and did not know they had positive blood Wassermans, 74.44 per cent showed a lymphocythemia; while in Group 4, the men who had received previous intravenous therapy, the percentage showing a lymphocytic increase was higher than in the other three groups, 82.60 per cent.

*The Polymorphonuclear Neutrophiles.*—In only 10 per cent of the 100 men with positive Wassermann reactions were the polymorphonuclear cells in the differential count above Howell's upper limit of the normal range, 75 per cent. Their percentage range above normal was highest in Group 1, men with initial lesions, 19.04 per cent; while in Group 2, men with skin manifestations, in none of the men were the polys above 75 per cent in the differential count; in Group 3, men with no symptoms or signs of syphilis, only 6.38 per cent of the cases showed a poly count above the upper limit of Howell's normal range; and in Group 4, men with previous treatment, 13.04 per cent showed a neutrophilia.

#### RECAPITULATION

Briefly the chief findings in the series of 100 differential blood counts on 100 nonallergic men with positive Wassermann reactions are:

First, that 40 per cent showed an eosinophilia ranging from 5 to 10 per cent.

Second, that in group 3, men *without* clinical symptoms and who did not know they had positive blood Wassermans, the percentage of men showing an eosinophilia was as high (44.68 per cent) as the men with primary lesions (42.85 per cent) and as the men with secondary lesions (44.44 per cent).

Third, that 74 per cent of the 100 men having positive reactions showed a lymphocythemia with a relative increase of the large lymphocytes.

#### HEMOGRAMS ON SIX MEN WITH POSITIVE (4-PLUS) WASSERMANN REACTIONS

Appreciating the daily variability of blood counts in health and disease, and realizing that our findings with only one differential count of 100 cells each in the four clinical groups totaling 100 men with positive Wassermann reactions are not unalterable conclusions, but may be regarded as only suggestive, a more

complete and intensive study was made on six men with strongly positive (plus 4) reactions, whose stools were negative for ova and parasites and who had received no previous medication. Eight differential counts were made in each case on separate days within a period of two weeks. These forty-eight counts of 100 cells each are tabulated in the modified Schilling hemograms (Tables I, II, and III).

TABLE I

## HEMOGRAMS ON SIX MEN WITH POSITIVE (PLUS 4) WASSERMANN REACTIONS

CASE 1.—W. P., colored, aged thirty-five years, married, one child. Wassermann plus 4, Jan. 2 and 5, 1933.

DATE	W.B.C.	GRANULAR LEUCOCYTES					LYMPHOCYTES			MONO-CYTES (TRANS.)
		YOUNG FORMS		MATURE FORMS			SMALL	LARGE	COMB.	
		MYELO.	BAND	POLY.	EOSIN.	BASO.				
1/14	6000	2	2	47	5	0	40	4	44	0
1/15		0	2	44	7	0	40	6	46	0
1/16		1	5	36	3	0	42	13	55	0
1/17		0	5	40	2	1	40	12	52	0
1/18		2	3	40	3	0	43	9	52	0
1/19	6400	0	3	42	10	0	44	1	45	0
1/22		1	1	40	12	0	44	1	45	1
1/24		0	2	40	2	0	45	11	56	0

CASE 2.—C. A., colored, aged nineteen years, single. Wassermann plus 4, Jan. 26, 1933.

2/ 3	5200	0	1	60	7	0	29	3	32	0
2/ 4		1	2	59	4	0	27	6	33	1
2/ 5		1	2	51	2	1	35	8	43	0
2/ 6		0	1	54	4	0	39	2	41	0
2/ 7		0	0	55	6	0	38	1	39	0
2/ 8	6400	1	1	50	9	1	33	4	37	1
2/ 9		0	0	62	3	0	29	6	35	0
2/10		0	0	49	5	0	34	10	44	2

TABLE II

## HEMOGRAM

CASE 3.—T. E., colored, aged thirty years, married, no children. Plus 4 Kahn. Feb. 2, 1933.

DATE	W.B.C.	GRANULAR LEUCOCYTES					LYMPHOCYTES			MONO- CYTES
		YOUNG FORMS		MATURE FORMS			SMALL	LARGE	COMP.	(TRANS.)
		MYELO.	BAND	POLY.	EOSIN.	BASO.				
2/ 2	8000	0	1	45	11	1	31	9	40	2
2/ 3		0	0	49	11	0	35	5	40	0
2/ 4		0	1	66	8	0	20	2	22	3
2/ 8		0	1	56	7	0	29	6	35	1
2/ 9		1	1	48	6	0	42	1	43	1
2/10	7400	1	3	50	7	1	30	5	35	3
2/15		2	5	53	6	0	29	4	33	1
3/ 3		0	1	53	13	0	31	2	33	0

CASE 4.—R. P., colored, aged forty-three years, married, one child. Plus 4 Wassermann. Jan. 8, 1933.

1/13	7000	1	3	50	1	1	31	13	44	1
1/14		0	4	61	3	0	25	7	32	0
1/15		0	1	72	2	2	19	4	23	0
1/16		4	2	42	5	0	40	7	47	0
1/17		1	2	48	2	0	42	5	47	0
1/18	5800	10	2	48	4	1	42	0	42	0
1/19		2	2	50	7	0	36	5	41	0
1/20		1	2	53	2	0	34	8	42	0

TABLE III

## HEMOGRAM

CASE 5.—W. B., white, aged twenty-two years, single. Wassermann plus 4, March 30, 1934.

DATE	W.B.C.	GRANULAR LEUCOCYTES					LYMPHOCYTES			MONO- CYTES
		YOUNG FORMS		MATURE FORMS			SMALL	LARGE	COMB.	(TRANS.)
		MYELO.	BAND	POLY.	EOSIN.	BASO.				
4/11	7400	1	0	46	8	0	38	3	41	4
4/12		0	0	44	10	0	40	5	45	1
4/13		0	0	43	7	0	43	3	46	4
4/14		1	1	38	2	1	48	3	51	6
4/15		0	0	35	6	1	50	4	54	4
4/16		1	0	37	4	0	44	12	56	2
4/17		0	0	50	4	0	39	2	41	5
4/18		0	0	47	9	0	37	3	40	4

CASE 6.—L. J., black, aged thirty-four years, married, one child. Wassermann plus 4, Mar. 30, 1934.

4/11		0	0	42	4	1	46	2	48	5
4/12		0	0	35	15	0	40	4	44	6
4/13		0	0	45	13	1	35	2	37	4
4/14		0	0	56	5	0	30	5	35	4
4/15		0	0	46	8	0	34	6	40	6
4/16		2	1	43	5	0	40	5	45	4
4/17		1	0	54	10	0	27	4	31	4
4/18	6800	0	0	46	4	0	37	6	43	7

## SUMMARY OF HEMOGRAM FINDINGS

The series of eight differential leucocyte counts made on each of six men with strongly positive (plus 4) blood Wassermann reactions, and tabulated in the modified Schilling hemograms, illustrates the importance of making repeated counts on separate days if the occurrence of eosinophilia is not to be overlooked. In each hemogram from two to eight of the counts showed the presence of a 5 to 15 per cent of *eosinophile* cells.

The findings in this smaller group on the whole confirm and tend to emphasize that the suggested conclusions drawn from the series of 100 positive cases may be considered on the average correct. Moreover, the counting of 100 cells on eight different days is probably of more value in substantiating the periodic occurrence of a blood eosinophilia than would have resulted from the counting of 800 cells in two or three blood smears made on the same day. It seems fair to presume, therefore, that had we made repeated differential counts, instead of only one, in the series of 100 men with positive reactions, the percentage of men showing a periodic occurrence of eosinophilia would have been much higher, possibly nearly 100 per cent.

The eight hemograms also show the uniform presence of an increase of the *lymphocytes* and correspond to a similar finding in the series of 100 cases. They confirm the reported findings in the literature of a lymphocytosis in syphilis. The increased proportional ratio of large to small lymphs is most evident in the hemogram counts and suggests a shift to the left, since the large lymphs are regarded by many hematologists as the younger form of cell.

The *neutrophilic granular polymorphonuclears* in the six hemograms are uniformly below the normal average. The younger forms of granular neutrophils, the myelocytes and band cells, are within normal limits, so that there

is no neutrophilic nuclear shift to the left. The large hyaline *monocytes* are also within the average normal range, so that no evidence of a monocytosis was present in any of the counts.

#### COMMENT

*The Occurrence, Possible Function and Significance of Eosinophilia.\**—In 1860 Wharton Jones<sup>11</sup> first distinguished finely and coarsely granular leucocytes in fresh blood. In 1878 Ehrlich<sup>12</sup> observed that the coarsely granular variety has special affinity for eosin stain, and since then an increased occurrence of so-called eosinophiles has been observed in a variety of conditions. Opie<sup>13</sup> reports the eosinophile as common to all mammalian species with an average number in man of 2 to 4 per cent. Sabin<sup>14</sup> has shown the percentage to vary from day to day and even from hour to hour.

*Clinically* eosinophiles first attracted attention and were regarded of diagnostic importance when they occurred in increased numbers in certain of the intestinal and tissue animal parasitic infections;<sup>15</sup> the toxins of the parasites were believed to cause disintegration of the polymorphonuclear cells with a change in staining quality of the granules. With advancing knowledge and research in hematology, an increased percentage of eosinophiles in the differential blood counts began to be reported in a large variety of conditions, including many skin diseases;<sup>15</sup> some of the acute exanthemas, especially scarlet fever,<sup>15</sup> and the postfebrile stage of measles; some forms of tuberculosis,<sup>16</sup> especially the intestinal variety; some cases of arthritis and rheumatism;<sup>17</sup> in some patients following the intravenous or intramuscular injection of the heavy metals,<sup>15</sup> mercury, bismuth, etc.; also following the injection of foreign proteins,<sup>18</sup> especially egg albumen, horse serum, and tuberculin;<sup>19</sup> and as shown by me<sup>20</sup> following intramuscular injections of venom (Crotalin) solution.

The occurrence of an eosinophilia in asthma<sup>21</sup> (at times to a marked degree<sup>16</sup>) had been noted for many years. Since about 1910, however, when Meltzer and Koessler independently first suggested the probable relationship of human hypersensitiveness and animal anaphylaxis, the presence of a greater or less degree of eosinophilia in allergic asthma has come to be regarded as a typical and a characteristic finding.

A *hypereosinophilia* occurs in some cases of Hodgkins' disease,<sup>15</sup> in splenomegaly, in some leucemias especially in the early stage of myelogenous leucemia, and cases of familial constitutional hypereosinophilia<sup>22</sup> have been reported. The occurrence of a hypereosinophilia in isolated, sporadic instances of widely different etiologic and varying pathologic conditions, is difficult to account for in our present state of knowledge, and may be regarded as more or less of a hematopoietic curiosity, interesting and worthy of mention. The constant appearance, however, of a moderate degree of eosinophilia (6 to 10 or 15 per cent) even if periodic, is a blood finding which occurs consistently enough, when searched for, in certain pathologic conditions and immunologic reactions to require an explanation.

\*The writer is indebted to Dr. Huston K. Spangler for reviewing the literature on eosinophilia.

For many years physiologists and hematologists regarded eosinophiles as disintegrated or dead polymorphonuclear cells without any special significance. Today in studying the morphology of blood cells there is a general agreement that the polymorphonuclear eosinophilic granulocyte is a distinct cell entity and has its origin in adult life chiefly from the bone marrow, as do the polymorphonuclear neutrophils. It has long been assumed that the specific function of the leucocyte depends upon the type of its granules. Why some granules take a neutral and others an acid or basic stain has never been satisfactorily explained.

The *neutrophilic granular leucocytes* possess ameboid movement and have phagocytic power. They contain a proteolytic ferment, are possessed of an intra- and extracellular (active in blood serum) digestive power, and are positively chemotactic for pyogenic cocci (streptococci, staphylococcus, pneumococcus, gonococcus, etc.) whose body substances contain protein material.

The *lymphocytes*, on the other hand, which are of lymphoid origin, have a relatively sluggish ameboid movement, are not phagocytic and elaborate a lipolytic (fat splitting) ferment possessed of only intracellular digestive power (not active in the blood serum) and evidence has been adduced showing them to be positively chemotactic for tubercle bacilli and treponemes whose bodies contain fatlike substances. The fat splitting ferments of the lymphocytes have been found most active in an acid medium.

The *eosinophiles* while of myelogenic origin like the neutrophils, possess less ameboid movement. Phagocytosis is rarely observed and eosinophiles have not been shown to produce proteolytic ferments as do the neutrophils, or lipolytic ferments as do the lymphocytes, but apparently they have some special function related to the disintegration and removal of foreign protein from the tissues and in combating the toxins of parasites and certain bacteria. With their acid-staining granules eosinophiles would be expected to be most active in acid medium as are the lymphocytes. Eosinophiles have been shown to be responsive to the toxins of tubercle bacilli and as indicated by the findings reported in the present investigation there is clinical evidence that the eosinophile is also responsive to the toxins of the *Spirocheta pallida*.

In recent years there has been a tendency to consider most eosinophilias, even those occurring in parasitic infections, skin diseases, the exanthemas, diphtheria and certain other microbial infections as a part of the general phenomena of allergy. The clinical and immunologic significance of a relative or actual increase of eosinophile cells in the blood has remained largely conjectural and without definite interpretation.

Weber<sup>23</sup> states: "Eosinophilia is to be regarded as a part of the general protective allergic reaction toward the presence in the body of foreign proteins or abnormal products of albuminous catabolic metabolism." Foster<sup>24</sup> does not believe that the foreign protein introduced as antitoxin is responsible for eosinophilia because in a large series of cases injected routinely with serums in a hospital for contagious diseases only those of diphtheria and tuberculosis produced an eosinophilic reaction. He thinks the toxins of the diphtheria and tubercle bacilli themselves exert a positive chemotaxis on the eosinophile cells of the

hematopoietic organs. Pepper and Farley<sup>1</sup> state that serum disease shows no eosinophilia, which would seem to support Foster's observation.

The finding of an eosinophilia in allergic conditions, in which antibodies have not thus far been demonstrable, might possibly be regarded as immunologic evidence that allergy and anaphylaxis are not of a similar mechanism. On the other hand, eosinophilia as a common finding in anaphylactic reactions in animals<sup>25, 26, 27, 28, 29</sup> and also in allergic diseases in man, would seem to be an indication of a related mechanism in the two conditions.

*Eosinophilia an Indication of Reactive Power of the Individual.*—In giving therapeutically more than 20,000 intramuscular injections of venom (crotalin) solution, with differential blood counts made before and after injections, to more than 1,000 patients (including cases of epilepsy, asthma, hay fever, eczema, arthritis, migraine, etc.), my experience has shown that in those cases in whom an increase in the percentage of eosinophiles does not occur in from three to six weeks following weekly injections, clinical improvement of the symptoms will be doubtful. In other words, an increase of eosinophile cells following crotalin injections may be used as an indication of the reactive power of the individual and has served in my hands as a guide to dosage.

The findings of Mueller, Otfried and Broesamlen<sup>30</sup> following the use of tuberculin, as a nonspecific agent, correspond to my experience with crotalin. They state their investigations indicate "that the increase in eosinophile cells following an injection was the surest sign of the reactive power of the patient against the toxin."

They made counts morning, noon, and night on the day preceding, the day of the injection and the day following injection. If after three injections there continued a nonappearance of the eosinophilia, the prognosis, as to the reactive power of the patient, was not good. Hence their conclusion was "that the behavior of the eosinophilic cells can be considered as a general standard of the reactive power of the organism in its struggle against infections."

The present study of differential blood counts in patients with positive Wassermann reactions tends to indicate that a moderate eosinophilia occurs, and is discoverable if repeated counts are made, at various stages of the patient's response to the specific toxin of the parasite of syphilis, in at least 40 per cent or more of the cases. It seems reasonable to assume, therefore, that the eosinophilia occurring in syphilis may be regarded as distinct evidence of the blood's reaction to the sensitizing (toxic) substance of the *Spirocheta pallida*, and that it is indicative of the patient's development of an allergic cellular mechanism of defense.

#### CONCLUSIONS

1. With the hypothesis that eosinophilia is a criterion of allergy and with the finding of an eosinophilia in individuals with positive Wassermann reactions in various stages of syphilitic infection, the possible immunologic relation of allergy and syphilis would appear to justify careful study.

2. With the admittedly high incidence of unrecognized syphilitic infection and the unknown basic factor of many allergic metabolic disturbances, any clue leading to a definite diagnosis and which may aid in solving the mechanism

involved in the systemic eosinophilogenic response, would seem worthy of attention and investigation from an immunologic standpoint.

3. Finally, it seems justifiable to conclude that a moderate degree of eosinophilia, especially when associated with a lymphocytosis, occurring even periodically in an apparently nonallergic individual whose intestinal tract is free from ova and parasites, warrants the taking of Wassermann tests and the making of a persistent search for clinical evidence of syphilis.

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# LABORATORY METHODS

## A SIMPLE MICROMETHOD FOR THE DETERMINATION OF ALCOHOL IN BIOLOGIC MATERIAL\*

R. N. HARGER, PH.D., INDIANAPOLIS, IND.

A LARGE number of investigators have proposed methods for the determination of alcohol in distillates from biologic material. The literature prior to 1927 has been summarized by Gettler and Tiber<sup>1</sup> and Fraenckel and Nicolai.<sup>2</sup> The chief developments since 1927 are the osmic acid method of Hiramatsu,<sup>3</sup> a modification of Stritar's alkyl iodide method by Gettler, Niederl and Benedetti-Pileher,<sup>4</sup> and Bock's application of the interferometer<sup>5</sup> as employed by Kionka and Hirsch.<sup>6†</sup>

Three methods are of the microtype. They are: the bichromate-iodide method of Widmark,<sup>7</sup> the alkyl iodide method of Gettler, Niederl and Benedetti-Pileher,<sup>4</sup> and the interferometer method. The first two are said to operate with as little as 0.1 mg. of alcohol, and in the interferometer method one scale division corresponds to 0.0005 per cent of alcohol. However, each method has certain rather serious drawbacks. In the Widmark method the starch-iodide end-point, using hundredth normal thiosulphate, is not very sharp. Gettler's ethyl iodide method is a gravimetric procedure and requires rather elaborate apparatus; also, an interferometer is expensive, and this method is invalidated by the presence of other dissolved material.

The following simple micromethod was developed in connection with a study of the metabolism of alcohol. This method operates with quantities of ethanol varying from 0.02 mg. to 0.5 mg. It is rapid and very simple, and has been in use in our laboratory and a few clinical laboratories during the past four years with entirely satisfactory results. In principle the method belongs to the bichromate group, and is a modification of certain methods referred to above.<sup>1, 2</sup> An excess of bichromate is employed, and, as shown by Derome and Pepin,<sup>8</sup> the heat developed upon adding the sulphuric acid (5 c.c. of sulphuric acid to 6 c.c. of the aqueous solution of alcohol and bichromate) is sufficient completely to oxidize the alcohol without the use of a water-bath. In titrating the excess of bichromate it was considered desirable to avoid time-consuming spot-tests or addition of excess of iodide or other reducing agent

\*From the Department of Biochemistry and Pharmacology, Indiana University School of Medicine.

†Since this paper was written the iodine pentoxide method of Haggard and Greenberg (*J. Pharmacol. & Exper. Therap.* 52: 137, 1934) has appeared. This method would seem to be rather complicated in that it requires one boiling to remove iodine, and three titrations,



and back-titration with thiosulphate or permanganate. After some experimenting it was found that this could be accomplished by using a reducing solution composed of methyl orange and ferrous sulphate. In the high concentration of sulphuric acid used in the procedure, methyl orange is rapidly decolorized by even minute quantities of bichromate. In fact, methyl orange alone may be used to titrate the excess of bichromate, but the end-point is not quite so sharp as when ferrous sulphate is also present to perform about 80 per cent of the reduction. The titration is carried out in the same concentration of sulphuric acid as was used for the oxidation of the alcohol by the bichromate, thus avoiding the usual dilution and transfer of the material; and the reaction vessel, an ordinary test tube, serves both for the oxidation of the alcohol and the titration of the bichromate. The end-point is very sharp. Finally, the high concentration of sulphuric acid in the red titration fluid raises the surface tension and specific gravity so that the size of drops is greatly decreased. Thus a moderately fine buret tip will deliver about 50 drops per c.c., which somewhat increases the accuracy of the method.

Methanol may also be determined by this method, in which case the oxidation proceeds to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ , whereas, with ethanol the alcohol is oxidized to acetic acid.

#### APPARATUS

*Reaction Tubes.*—Ordinary test tubes with inside dimensions of about 17 mm.  $\times$  150 mm. are satisfactory.

*Stirring Rod.*—A glass rod ending in a 12 mm. ring, whose plane is at right angles with the rod.

*Microburet.*—A 5 c.c. microburet, which should be standardized.\* To prevent the buret contents from coming in contact with the suction tube when filling from the tip, a bulb with bent tube was sealed at the top.

*Device for Stirring with an Air Current During the Titration.*—The stirring may be done with the stirring rod but this is rather tiresome when analyzing many samples and we prefer the following arrangement (see Fig. 1):

The tip of the microburet is drawn out to form a tube about 2.5 mm.  $\times$  35 mm. with the lower end constricted to about 1.2 mm. A rubber sleeve with a glass side arm is made from a  $1\frac{1}{4}$  in. length of  $\frac{5}{16}$  in. rubber tube which is punctured about midway with a small hole. A short length of small glass tubing, flanged at one end and tapered at the other, is pushed through this hole from the inside until the flange is flush with the inner wall of the rubber tube. The rubber sleeve is now pushed over the drawn-out nozzle of the buret until the tip of the nozzle projects about  $\frac{7}{8}$  inch below the bottom of the sleeve and the upper end of the sleeve makes a tight joint with the nozzle. The glass side arm is connected with the suction pump by means of fine flexible rubber tubing running to a bent glass reducer supported by the ring stand holding the buret. A two-hole rubber stopper, which fits the reaction tube, carries in one hole a bubbler tube terminating in a bulb with small holes for breaking up the air current and in the other hole a piece of thin-walled glass tubing about 4.5 mm. wide and 18 mm. long.

\*Empire Laboratory Supply Co. No. 3137 is satisfactory.

In making a titration the reaction tube is closed by means of the rubber stopper carrying the bubbler tube and is placed beneath the buret and raised so that the short glass tube is pushed over the buret tip and into the rubber sleeve until a tight joint is made and the buret tip extends about  $\frac{1}{2}$  inch below the lower end of the glass tube. The buret is now lowered until the reaction tube is supported on the table. A screw clamp regulates the air flow to the suction pump so that the liquid is well stirred during the titration. At the end of the titration the reaction tube and its rubber stopper are removed from the buret, and the rubber stopper and its bubbler tube transferred to the next tube to be titrated.

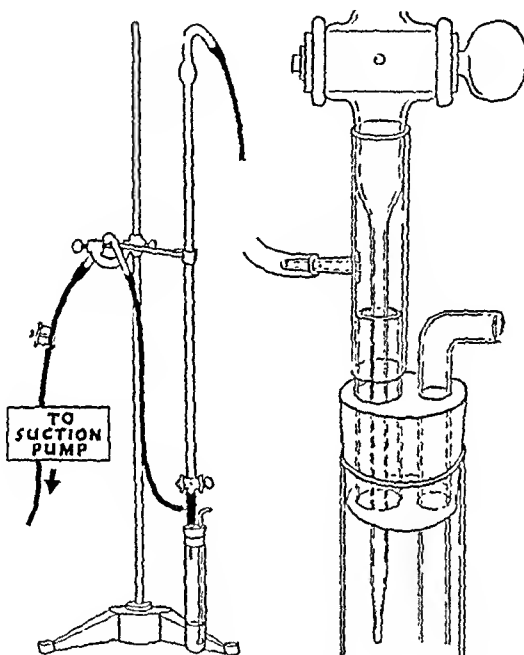


Fig. 1.—Device for stirring with an air current when many samples are to be titrated.

#### REAGENTS

*Concentrated Sulphuric Acid.*—A good grade of C. P. sulphuric acid containing very little reducing substance is employed.\*

*Sulphuric Acid (62%).*—Pour one volume of concentrated C. P. sulphuric acid into an equal volume of water and cool.

*Methyl Orange (0.1%).*—Dissolve one gram of methyl orange in one liter of approximately N/40 NaOH and filter. This will keep indefinitely.

*Ferrous Sulphate (20%).*—Dissolve 50 grams of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  in 150 c.c. of water. Add 30 c.c. of conc.  $\text{H}_2\text{SO}_4$  and dilute to 250 c.c. In a stoppered flask this solution will undergo little oxidation for one year.

*Red Reducing Fluid.*—Place in a flask 35 c.c. of the 62 per cent sulphuric acid. Add to this 15 c.c. of the methyl orange solution and 1 c.c. of the fer-

\*Baker & Adamson's C. P. Sulphuric Acid, No. 1180, gave very small blanks.

rous sulphate solution, shaking the flask during the additions. Mix well and cool to room temperature. This solution deteriorates slowly but will keep for three or four days, being more rapidly decomposed in warm weather. About 2.5 c.c. of this solution are required for each cubic centimeter of the standard bichromate solution.

*Standard Bichromate Solution, 0.0131 N.*—Dissolve 2.129 gm. of C. P. potassium bichromate in water and make up to one liter. One cubic centimeter of this solution is equivalent to 0.5 mg. of ethanol or 0.232 mg. of methanol.\* The bichromate may be standardized against a weighed amount of anhydrous ethanol or in the manner described in standard works on quantitative analysis, but we have found that a good grade of C. P. analyzed bichromate (Baker and Adamson or J. T. Baker) is sufficiently pure for use as a primary standard.

#### REMOVAL OF ALCOHOL FROM BIOLOGIC MATERIAL

*Tissues.*—The material is finely hashed, mixed with an equal weight of water and acidified with a little tartaric acid. It is now steam distilled until the volume of distillate equals the weight of tissue used. One cubic centimeter of this distillate is diluted to 50 c.c., and 5 c.c. of the diluted material, representing 0.1 gm. of tissue, is used for the analysis. Where the tissue alcohol is less than 0.05 per cent the dilution of the distillate should be less than 1:50. If the tissue alcohol is above 0.5 per cent, one should use less than 5 c.c. of distillate, adding enough water to make 5 c.c.

*Blood.*—Since blood foams badly when steam distilled, much time is saved by first preparing a protein-free filtrate and distilling a portion of this. It was found that results by this process agree well with those obtained by steam distilling the blood. A tungstic acid protein-free filtrate of the blood is prepared by the Folin-Wu method.<sup>9</sup> Five cubic centimeters of this filtrate are placed in a 125 c.c. Pyrex distilling flask. To this is added 20 c.c. of water and some glass beads. The flask is connected to a small vertical condenser, the lower end of which extends well into a Folin receiving tube graduated at 25 c.c. The distilling flask is heated directly over a small flame from a microburner which is protected by a mica chimney. Distillation is continued until about 12 c.c. have come over, which requires about five minutes. The condenser tube is then rinsed out with a little water and the rinsing water collected in the receiving tube, after which the contents of the receiving tube are made up to 25 c.c. Five cubic centimeters of this distillate, representing 0.1 c.c. of blood, are used for the analysis. If the alcohol is above 0.5 per cent or below 0.05 per cent, the procedure is modified as described under "Tissues." If this distillate is to be kept some hours before analysis, it should be acidified with about six drops of concentrated  $H_2SO_4$ .

*Urine.*—The urine is diluted 1:10. From this point the procedure is identical with that used for a protein-free filtrate of the blood.

\*Acetone causes a slight reduction of the bichromate in this method, 5 mg. of acetone consuming 0.6 c.c. of the dichromate.

## ANALYSIS OF DISTILLATES

In a reaction tube place 5 c.c. of the solution of alcohol from the distillation of blood, urine or tissues, which should contain not more than 0.5 mg. of ethanol or 0.23 mg. of methanol. Add 1 c.c.\* of the standard bichromate solution and then 5 c.c. of the concentrated sulphuric acid. Mix well by means of the stirring rod and allow to stand for ten minutes. Cool in water to room temperature and titrate the excess of bichromate with the red reducing fluid. The first permanent pink color should be the end-point. In the same manner run a blank on 5 c.c. of distilled water. Since sulphuric acid usually contains a trace of reducing material, the correct figure for expressing relationship of the red reducing fluid in terms of bichromate is obtained as follows: After titration add to one of the tubes a second cubic centimeter of the standard bichromate, mix the contents well and again titrate.

## CALCULATION

U = titration figure for unknown.

W = titration figure for distilled water.

B = titration figure for extra c.c. of bichromate.

Q = quantity of blood, urine, or tissue represented by the aliquot analyzed.

Then,  $\frac{W - U}{B} \times 0.5 \div Q =$  mg. of ethyl alcohol, per c.c. of blood or urine or gram of tissue. Where  $Q = 1/10$  the formula becomes  $\frac{W - U}{B} \times 5$ . (For methanol use 0.23 instead of 0.5.)†

## RESULTS WITH THE METHOD

Seventeen dilutions of absolute ethanol in water were analyzed by a student, the analyses being done in duplicate. The results are given in Table I.

TABLE I  
RESULTS WITH THE METHOD (ETHANOL)\*

SAMPLE	ETHANOL USED (MG. IN 5 C.C.)	ETHANOL FOUND (MG. IN 5 C.C.)	
		(A)	(B)
1	0.431	0.430	0.438
2	0.416	0.431	0.437
3	0.357	0.349	0.357
4	0.250	0.263	0.263
5	0.200	0.196	0.193
6	0.166	0.157	0.157
7	0.147	0.145	0.131
8	0.143	0.144	0.131
9	0.110	0.103	0.110
10	0.074	0.078	Lost
11	0.050	0.054	0.049
12	0.042	0.049	0.044
13	0.030	0.034	0.036
14	0.020	0.023	0.025
15	0.010	0.009	0.003
16	0.010	0.012	0.008
17	0.000	0.000	0.000

\*Composition of samples unknown to analyst.

\*Where the sample analyzed contains less than 0.05 mg. ethanol, the accuracy is improved by using less bichromate (0.5 c.c. or 0.2 c.c.) and reducing the amount of ferrous sulphate in the red titration fluid to 0.5 c.c. or 0.2 c.c.

†Normal blood or tissues show a small amount of reduction by this method, giving a blank of 0.02 to 0.04 c.c. of 0.0434 N  $K_2Cr_2O_7$  for a 0.1 gm. sample. Part of this reduction is due to a volatile material in the distilled water used for diluting purposes which is concentrated in the process.

TABLE II  
RESULTS WITH THE METHOD (METHANOL)\*

SAMPLE	METHANOL USED (MG. IN 5 C.C.)	METHANOL FOUND (MG. IN 5 C.C.)	
		(A)	(B)
1	0.200	0.200	0.205
2	0.167	0.175	0.195
3	0.143	0.146	0.147
4	0.120	0.125	0.121
5	0.100	0.103	0.105
6	0.067	0.071	0.068
7	0.046	0.043	0.048
8	0.018	0.022	0.014
9	0.009	0.008	0.008
10	0.000	0.004	0.002

\*Composition of samples unknown to analyst.

The method was also applied to aqueous solutions of methanol with the results given in Table II.

#### SUMMARY

1. A micromethod is described for determining alcohol in biologic material. This method operates satisfactorily with quantities of ethanol varying between 0.5 mg. and 0.02 mg.

2. The procedure is a modified bichromate method, the excess of bichromate being titrated directly with a mixture of ferrous sulphate and methyl orange.

3. The method employs very simple apparatus and consumes about as much time as a determination of blood sugar.

4. For convenience, where many analyses are required, a device is described for stirring with an air current during the titration.

5. The method will also determine methanol.

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## THE STABILITY OF SUGAR IN THE CEREBROSPINAL FLUID<sup>2</sup>

PURCELL G. SCHUBE, M.D., BOSTON, MASS.

THE cerebrospinal fluid sugar has been investigated until its estimation is considered of sufficient value in the diagnosis of certain pathologic conditions to become a routine procedure in many laboratories. It has been felt generally that the estimation of this sugar should be performed at once; that otherwise the sugar would decrease in amount and the estimation become of little accurate value.

It is the purpose of this paper to test the validity of this "general feeling."

### METHOD

The cerebrospinal fluid was drawn into clean, dry, sterile tubes which were stoppered and placed in an ice box at 10° C. where they remained through the period during which the estimations were made. When a quantity of fluid was desired for a sugar estimation, the tube was unstoppered and a small amount, sufficient for the estimation, was poured into another tube, thereby eliminating the necessity of sterile pipettes. The original tube was restoppered and placed in the ice box. The sugar was determined by treating the cerebrospinal fluid with 0.5 c.c. of 10 per cent sodium tungstate solution and then with 0.5 c.c. of 2/3 N sulphuric acid to precipitate the protein. After centrifugation the clear protein-free supernatant fluid (a 1:1 dilution of the original spinal fluid) was used for the determination of its sugar content by the Folin-Wu blood sugar procedure,<sup>1</sup> using for colorimetric comparison a Bausch and Lomb Duboseq colorimeter. The sugar estimations were run in duplicate, the average of the two estimations being used in each instance. The sugar content was expressed as milligrams of glucose per 100 c.c. of spinal fluid.

The determination on the cerebrospinal fluid sugar was done immediately after the fluid had been obtained and cooled to 10° C., and then on the following third, fifth, seventh, tenth, fourteenth, and twenty-first days. The results obtained are presented in Table I.

### DISCUSSION

In this study the cerebrospinal fluids of 24 unselected individuals were used. These individuals all presented neuropsychiatric problems with and without demonstrable organic pathology. The only criterion determining the use of a fluid was its sterility. This requisite was considered as justified, and contaminated fluids were discarded.

<sup>1</sup>From the Psychiatric Clinic, Boston State Hospital.  
Received for publication, August 17, 1934.

Throughout the period of study of these fluids, twenty-one days, the variation of the obtained values was entirely within that of the experimental method used. The largest average variation of the sugar values was 1.8 mg. and the smallest, 0.4 mg. The largest range of variation was 3.0 mg. and the smallest, 0.6 mg.

TABLE I  
SPINAL FLUID SUGAR\*

CASE	ON DAY PUNCTURE PERFORMED	3RD	5TH	7TH	10TH	14TH	21ST	AVERAGE	AVERAGE VARIATION	VARIATION RANGE
1	60.7	60.4	60.9	61.0	60.3	60.7	60.8	60.7	0.4	0.7
2	42.8	43.0	42.1	42.7	43.2	42.6	43.0	42.7	0.5	1.1
3	68.8	68.5	68.0	67.9	68.8	69.3	69.0	68.6	0.7	1.4
4	50.6	52.0	51.5	51.7	50.2	50.0	49.8	50.8	1.2	2.2
5	53.2	53.6	54.0	52.2	53.2	52.0	53.5	53.0	1.0	2.0
6	47.5	46.4	48.0	48.5	47.5	46.8	47.0	47.3	1.2	2.1
7	62.8	62.4	62.8	63.2	63.0	63.0	62.6	62.8	0.4	0.8
8	66.1	63.5	66.1	64.2	64.0	66.5	66.0	65.2	1.3	3.0
9	57.3	57.3	57.2	56.8	55.7	56.0	56.7	56.7	0.6	1.6
10	50.6	50.4	51.2	51.4	52.0	51.8	51.4	51.2	0.8	1.6
11	48.0	48.0	48.0	46.5	47.2	46.0	46.0	47.1	0.9	2.0
12	54.4	52.8	53.6	52.0	52.0	52.2	53.2	52.8	1.6	2.4
13	55.0	55.0	56.2	54.8	54.6	55.2	55.0	55.1	1.1	1.6
14	62.7	62.7	62.7	62.7	60.2	61.5	62.0	62.0	0.7	1.2
15	56.7	58.5	57.3	58.8	56.7	57.0	57.0	57.4	1.4	2.1
16	67.1	67.2	65.0	66.4	67.5	67.3	67.0	66.7	0.8	2.5
17	60.5	61.2	61.2	60.4	60.8	60.0	61.8	60.8	1.0	1.8
18	55.4	53.8	54.6	53.0	53.2	54.0	55.0	54.1	1.3	2.2
19	53.2	54.0	53.6	52.0	53.2	53.2	53.2	53.2	0.8	2.0
20	67.2	67.2	67.4	65.4	65.6	66.0	66.5	66.4	1.0	2.0
21	50.8	51.2	50.4	51.2	50.8	50.8	50.6	50.8	0.4	0.6
22	62.0	62.4	63.2	61.6	62.0	63.2	63.5	62.5	1.0	1.9
23	64.4	65.5	63.6	62.6	62.8	63.4	64.0	63.1	1.8	2.9
24	56.8	59.2	59.0	57.6	56.8	58.0	57.5	57.8	1.4	8.4

\*Expressed in mg. per 100 c.c. cerebrospinal fluid.

Therefore, from a study of the results presented, the conclusion can be drawn that: if after the cerebrospinal fluid is obtained it is kept under sterile conditions and at a temperature of 10° C., the amount of change of the sugar therein over a period of at least twenty-one days is entirely negligible.

#### CONCLUSIONS

The quantity of sugar in cerebrospinal fluid kept under sterile conditions at 10° C. remains unchanged for a period of at least twenty-one days.

#### REFERENCE

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## ANTIGEN MIXING TUBE FOR THE KAHN TEST\*

ROBB SPALDING SPRAY, PH.D., MORGANTOWN, W. VA.

THE Kahn test has been accepted as one of the standard laboratory procedures, and has been widely adopted. One of its outstanding virtues is its adaptability in the small laboratory. In such a situation one has frequent occasion, or demand, to run at a given time only a single test, or at most from 3 to 5 tests.

The Kahn antigen, whether purchased or made in the laboratory, is a relatively expensive reagent, and the device herein described has, among other advantages, that of reducing the cost of antigen by one-half when but few serums are to be tested.

The original Kahn method requires the use of a minimum of 1 c.c. of antigen to the titrated amount of saline. These are mixed in shell vials by pouring rapidly back and forth. Due to the necessity of rapid mixing, Kahn advocates this minimum amount of antigen.

In the hands of the inexperienced, however, one frequently spills a portion, or hesitates in the process, with the resultant precipitation of the cholesterol. In the device described here the two reagents are placed in the same tube, in separate compartments, and the mixing is effected by tilting the tube and shaking. Since there is no possible loss, nor any lag in time of mixing, smaller quantities of antigen may be used.



The tube may be simply made in any laboratory having a blast burner, or even the Fisher type of burner. One takes a standard 8 by 1 inch test tube and heats the bottom of the tube, holding the tube horizontally in the flame, until well softened. Then, by cautiously blowing, a small convex blister is formed on the side of the tube just at the end. After this has cooled one heats the tube again about 1 inch higher up and blows a second pocket parallel to the first. When cooled the tube is cut off about 3 inches long.

Several attempts may be required before a satisfactory result is obtained. Each pocket should hold approximately 1 c.c. of fluid when the tube is held almost horizontal, with the pockets downward.

In use the antigen is pipetted into the bottom pocket, the saline into the upper pocket. The tube is then upended and instantly shaken, or struck against the other hand, for ten seconds. The antigen mixture is then drained into a shell vial and allowed to stand ten minutes before using.

This device has been in use in our laboratory for nine years with perfect satisfaction. We find that the use of 0.5 c.c. of antigen with the usual amount of saline (titer 1:1.1 or 1.2 c.c.) 0.55 or 0.6 c.c. gives sufficient for 7 or 8 tests.

\*From the Department of Bacteriology, Medical School, The West Virginia University.  
Received for publication, September 29, 1934.



This procedure thus saves one-half the antigen, which, over the course of a year, means a considerable saving in money.

A sketch of the device is appended to illustrate its simplicity.

## DETERMINATION OF NITRATE IN ANIMAL TISSUES\*

MARY WHELAN, M.A., OKLAHOMA CITY, OKLA.

IN ORDER to investigate the fate of nitrate compounds taken into the body, it was necessary to find a method suitable for analysis of animal tissues. Pucher, Vickery, and Wakeman<sup>1</sup> have devised a method for the determination of nitric acid in plant tissues. Their method was adapted, with certain modifications, and found to give satisfactory results when applied to animal tissues.

*Preparation of Material.*—The tissues were removed from the body as quickly as possible, made alkaline with 10 per cent sodium hydroxide, and dried in a drying oven, or on a steam bath. After the tissues were completely dry they were ground in a small food grinder.

*Procedure.*—One gram of finely ground dry tissue in a 100 c.c. beaker was mixed with sufficient 4 N sulphuric acid to bring the pH below 1. Since it was not practical for us to determine the pH of each tissue, varying quantities of acid were added to 2-gm. samples, and an analysis of each made to determine the quantity of acid necessary, and also to determine whether more than the exact amount of acid required to adjust the pH would in any way affect the results. Table I shows that at least 3 c.c. of 4 N acid are required and that amounts beyond this give the same results. It would seem that the tissue must be made distinctly acid but that an accurate adjustment of the pH is not necessary.

TABLE I

WEIGHT OF SAMPLE GM.	4N H <sub>2</sub> SO <sub>4</sub> C.C.	NITRATE N MG.
2	1	0.0243
2	2	0.0775
2	3	0.54
2	4	0.48
2	5	0.59
2	6	0.58
2	10	0.585

The tissue and acid are mixed and 2.5 gm. of pure asbestos fiber are added, and the whole stirred to insure a thorough mixing. A 26 by 60 mm. Schleicher and Schüll paper extraction thimble is placed in a siphon tube (Eimer and Amend No. 30,754). A glass rod is placed in the siphon tube to hold the thimble away from the wall on one side. The transfer of material is simplified by using a 10 cm. funnel the stem of which has been cut off. The funnel fits

\*From the Department of Biochemistry and Pharmacology, School of Medicine, University of Oklahoma.  
Received for publication, September 13, 1934.

TABLE II

TISSUE	DOG	EXTRACTION 1 COLORIMETRIC DE- TERMINATION		EXTRACTION 2 COLORIMETRIC DE- TERMINATION		EXTRACTION 3 COLORIMETRIC DE- TERMINATION	
		1	2	1	2	1	2
Brain	1	0.98	1.20	0.97	0.97		
	2	1.00	1.00	1.00	1.00		
	3	0.50	0.50	0.70	0.70		
	4	0.50	0.50	0.63	0.63		
Spinal cord	1	1.44	1.40	1.43	1.30		
	2	1.26	1.26	1.33	1.33		
	3	1.00	0.97	0.93	0.93		
	4	0.82	0.82	0.90	0.90		
Trachea	1	1.33	1.83	1.80	2.00		
	2	1.47	1.47	1.63	1.63	1.38	1.38
	3	0.57	0.57	0.44	0.43		
	4	0.40	0.40	0.43	0.43		
Lung	1	0.97	0.95	0.97	0.97		
	2	1.14	1.26	1.13	1.27		
	3	0.48	0.48	0.57	0.60		
	4	0.83	0.80	0.60	0.70		
Stomach	1	1.13	1.13	1.17	1.17		
	2	0.96	0.90	0.84	0.84	0.80	0.76
	3	0.34	0.30	0.40	0.43		
	4	0.77	0.77	0.60	0.60		
Pancreas	1	1.50	1.49	1.67	1.65		
	2	1.60	1.58	1.60	1.62		
	3	0.57	0.57	0.60	0.60		
	4	0.60	0.57	0.47	0.47		
Spleen	1	1.80	2.00	2.02	2.00		
	2	1.49	1.50	1.40	1.40		
	3	0.40	0.40	0.39	0.41		
	4	0.60	0.60	0.66	0.66		
Kidney	1	1.10	1.10	1.10	1.30		
	2	1.30	1.36	1.50	1.50	1.46	1.47
	3	0.70	0.60	0.63	0.63		
	4	0.80	0.80	0.67	0.67	0.83	0.83
Intestine	1	1.00	1.05	1.00	1.00		
	2	0.76	0.76	0.60	0.60		
	3	0.56	0.56	0.70	0.70		
	4	0.30	0.30	0.40	0.30		
Muscle	1	1.77	1.67	2.00	2.00		
	2	1.30	1.33	1.36	1.27	1.47	1.47
	3	0.80	0.79	0.80	0.87		
	4	0.70	0.70	0.76	0.80		
Esophagus	1	1.23	1.20	1.27	1.27		
	2						
	3	1.33	1.33	1.07	1.10		
	4						
Skin	1	1.435	1.47	1.50	1.43		
	2	1.130	1.10	1.10	1.10		
	3	0.80	0.75	0.80	0.75		
	4	0.96	0.94				
Bladder	1	1.14	1.24	1.14			
	2	1.28	1.28	1.27			
	3	1.10	1.10				
	4	Lost					

nicely into the mouth of the thimble. The small amount of material which clings to the sides of the beaker and funnel is removed with a small piece of cotton, which is then used as a plug for the thimble. The siphon tube is attached to the spiral metal condenser of the extraction apparatus. The whole is then placed in the flask which contains 150 or 200 c.c. alcohol-free ether, and a few quartz pebbles. The apparatus is placed on an electric hot plate and the extraction conducted for eight hours. At the end of that time about 75 c.c. of hot distilled water are poured through the thimble into the flask. A few drops of phenolphthalein are added and the solution made alkaline with 10 per cent Na OH. The ether is then distilled off slowly. The residue is transferred to a 100 c.c. volumetric flask and made up to volume. Seventy-five cubic centimeters of this are transferred to a porcelain evaporating dish and evaporated to dryness. The residue is moistened with a few cubic centimeters of water, a few drops of concentrated sulphuric acid added to make the solution distinctly acid, and the whole transferred to a 25 c.c. volumetric flask. Ten cubic centimeters of 5 per cent mercuric chloride are added and the solution made up to volume by washing the evaporation dish with water, a few cubic centimeters at a time. The mercuric chloride precipitates all interfering substances and the filtrate is clear, and, except in the case of highly pigmented tissues, quite colorless. The small amount of color present does not interfere with the development or reading of the blue color produced with diphenylbenzidine. The amount of nitrate present is determined by the method of Whelan.<sup>2</sup>

#### DISCUSSION AND CONCLUSIONS

The detailed results of analysis made with this method, as a part of a study reported in another paper, are given in Table II. In all cases extractions were made in duplicate, and in some instances in triplicate. And in all cases the colorimetric determinations were made in duplicate. As the table shows, the results are close and indicate that the method determines the amount of available nitrate present.

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## A MICRO-KJELDAHL TECHNIC FOR DETERMINING FIBRINOGEN\*

HELEN R. GARBUTT, A.B., AND ROGER S. HUBBARD, PH.D., BUFFALO, N. Y.

IN A previous article<sup>1</sup> a technic was described for determining albumin and globulin in serum in which a micro-Kjeldahl oxidation and subsequent nesslerization in the presence of Rochelle salts were used. This method was quite convenient and sufficiently accurate for ordinary diagnostic purposes. It was deemed desirable to develop a similar method for determining fibrinogen, for this analysis is not technically as satisfactory as are most of those in use in diagnostic laboratories. The main objection to an application of the methods already available<sup>2</sup> lay in transferring the fibrin formed by the action of calcium chloride to the tube in which the micro-oxidation was to be carried out. Neither of the two procedures commonly recommended for this purpose seemed quite satisfactory. In the simultaneous destructive oxidation of filter paper and precipitate by the Folin and Wu reagent, the presence of such a large amount of carbon was undesirable, and collection of the precipitate on a glass rod and its subsequent transference to the test tube seemed to furnish opportunities for loss which should be avoided if it were possible to do so. These difficulties led us to experiment with precipitation in centrifuge tubes, and to study the most suitable method for carrying through the oxidation in the same tubes. A few minor changes in technic were found either necessary or convenient for carrying out the procedure in a satisfactory manner, and the method described below, which, in the opinion of the authors, is a very convenient one, and of a degree of accuracy well adapted to diagnostic work, was developed.

### DESCRIPTION OF METHOD

*Apparatus.*—(1) Conical 50 c.c. centrifuge tubes of Pyrex glass graduated at 10 c.c., (2) microburner, and (3) ring stand with adjustable clamp to hold centrifuge tubes.

*Reagents.*—(1) Eighty-five hundredths per cent sodium chloride, (2) 2.5 per cent calcium chloride, (3) 0.05 per cent calcium chloride, (4) the dilute 1:1 sulphuric acid and phosphoric acid digestion mixture of Folin and Wu,<sup>3</sup> (5) 10 per cent Rochelle salts, (6) Nessler's solution prepared according to the directions of Folin and Wu,<sup>3</sup> (7) a strong ammonium sulphate standard containing 0.1414 gm. of pure salt in 100 c.c. of water, and (8) a weak ammonium standard prepared by diluting 10 c.c. of the one just described to 100 c.c.

*Method.*—Place 0.5 c.c. of plasma in a Pyrex centrifuge tube graduated at 10 c.c. Add 24 c.c. of 0.85 per cent sodium chloride and 0.5 c.c. of 2.5

\*From the Buffalo General Hospital.

Received for publication, September 13, 1934.

per cent calcium chloride solution. (These are the reagents recommended by Cullen and Van Slyke.<sup>4</sup>) Let stand until the clot has formed, which usually takes about an hour. Centrifuge at high speed until the clot has packed into the bottom of the tube. Decant the supernatant liquid. Wash twice with 5 c.c. portions of 0.05 per cent calcium chloride solution. Add 2 c.c. of the dilute oxidizing mixture of Folin and Wu containing sulphuric and phosphoric acids. Heat the tube over a low flame, shaking constantly to prevent bumping, until the precipitate has dissolved in the digestion mixture. Clamp the tube in a slanting position and heat carefully with a microburner until all the water has been driven off and white fumes appear. Then place the tube in a vertical position, cover with a watch glass, and continue heating until oxidation is complete and the contents of the tube have become colorless. Let cool until the fumes have almost wholly subsided, then tilt the tube and dilute with distilled water from a medicine dropper, pouring the water carefully down the side of the tube, adding only a drop at a time at first and more rapidly later. Let cool. If a crystalline precipitate has formed at this point, it may be broken up with a glass rod and the rod rinsed into the tube. Make the volume to 10 c.c., and centrifuge if necessary. Transfer 5 c.c. into a second tube graduated at 10 c.c., add 1 c.c. of 10 per cent Rochelle salts, make up to 10 c.c., and nesslerize with 15 c.c. of the reagent described by Folin and Wu. Simultaneously nesslerize standard solutions containing known amounts of ammonium sulphate, 1 c.c. of the dilute oxidizing reagent of Folin and Wu, and 1 c.c. of 10 per cent solution of Rochelle salts in a total volume of 10 c.c. Convenient standards contain 0.15, 0.09, and 0.06 mg. of nitrogen. These amounts are contained in 5, 3, and 2 c.c. of the dilute standard described above. Compare the color of the unknown solution with that of the standard nearest to it in tint in a colorimeter, and calculate the percentage of the fibrinogen present by means of the following formula:

$$\text{Per cent fibrinogen} = \frac{\text{reading of standard}}{\text{reading of unknown}} \times \text{value of standard} \times \frac{400}{1000} \times 6.25$$

#### STANDARDIZATION OF METHOD

Various experiments were carried out in attempting to standardize the method. The accuracy of the oxidation technic was established by precipitating proteins from blood serum and from alkaline solutions of various kinds (egg white, pollens, etc.) and comparing the results obtained by the direct procedure described with those given by the method of Hubbard and Sly.<sup>1</sup> The agreement in these experiments was very satisfactory.

The probable efficacy of washing with calcium chloride as a method of separating the precipitated protein from interfering nitrogenous compounds was also investigated. It was found that one treatment with calcium chloride was probably sufficient, for no nitrogen was found in the supernatant fluid from subsequent washings, and the nitrogen content of the precipitate after one, two, and three washings was identical. Nevertheless, to guard against possible error, it has seemed best to recommend the double treatment described.

As a convenient method of testing the accuracy of the method, we made a comparison between fibrin determinations made as described and studies based upon the difference between the total nitrogen content of plasma and of plasma from which fibrinogen had been removed.<sup>5</sup> The results of these studies were quite unsatisfactory. There was a fair degree of parallelism between the results of the two methods, but individual values often differed markedly from each other. In some experiments the determination described in this paper gave the higher value, and in others the reverse was true. The explanation of the cause of these discrepancies was obvious, for the actual value of the difference upon which the comparison was based was often very little greater than the algebraic sum of the errors inherent in the two micro-Kjeldahl analyses upon the treated and untreated plasma.

It was, therefore, necessary to adopt some other plan for testing the probable accuracy of the proposed method, and a comparison of results on varying amounts of plasma was accordingly made. In these studies no variations were introduced into the procedure, except that the total volume of salt plus plasma was kept constant and that the number of standards prepared was increased to allow for the large range of nitrogen values encountered. The results of these experiments are given in Table I.

TABLE I

VALUES OBTAINED IN DUPLICATE DETERMINATIONS ON VARYING AMOUNTS OF PLASMA

(Values expressed as gm. fibrinogen per 100 c.c. plasma)

SERIAL NO.	1 C.C. TAKEN	0.5 C.C. TAKEN		0.25 C.C. TAKEN
Plasma 1	--	0.53%	0.53%	--
2	--	0.69%	0.53%	--
3	--	0.43%	0.43%	--
4	--	0.57%	0.56%	--
5	--	0.36%	0.35%	--
6	--	0.42%	0.43%	--
7	--	0.89%	0.87% 0.91%	--
8	0.43%	0.42%		0.50%
9	0.20%	0.21%		--
10	0.56%	0.51%		0.51%
11	0.27%	0.30%		0.28%
12	0.42%	0.39%		0.41%
13	0.44%	0.44%		0.41%
14	--	0.56%		0.56%
15	0.36%	--		0.37%
16	0.43%	--		0.42%
17	0.23%	0.22%		0.23%
18	0.65%	0.57%		0.59%
19	0.60%	0.50%		0.55%
20	0.53%	0.46%		--
21	0.35%	0.35%		--
22	0.23%	0.24%		0.23%
23	0.29%	0.29%		0.27%
24	0.41%	0.41%		--
25	0.23%	0.23%		--
26	0.35%	0.35%		0.31%
27	0.37%	0.37%		0.35%
28	0.52%	0.56%		--
29	0.14%	0.14%		0.14%
30	0.11%	0.11%		--
31	0.46%	0.48%		0.48%
32	0.33%	0.33%		--

TABLE II  
PER CENT DIFFERENCE BETWEEN PAIRS OF VALUES

PAIRS COMPARED	RANGE OF PER CENT DIFFERENCE				
	0 TO 2% NO.	3% TO 5% NO.	6% TO 10% NO.	10% TO 15% NO.	OVER 15% NO.
0.5 with 0.5 c.c.	6	2	1	—	—
0.5 with 1.0 c.c.	11	4	4	2	1
0.5 with 0.25 c.c.	5	4	4	2	—
0.25 with 1.0 c.c.	3	5	5	2	—
Total	25	15	14	6	1

It is evident that in general the agreement shown by these determinations was quite satisfactory. Table II has been prepared to show just how close this agreement was. In this table is given the number of pairs agreeing closely together, those showing differences between 2 and 5 per cent, etc. The table shows that even under these rather extreme conditions the majority of the pairs of determinations showed differences of less than 5 per cent. The average of all the values was 4.3 per cent. Such a degree of accuracy is sufficiently great for a method designed for use in a diagnostic laboratory.

#### CONCLUSION

A method of determining fibrinogen, which renders transference of the precipitated material unnecessary, is described. The method gave results having a degree of accuracy sufficiently great to justify its use in routine work.

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## A NEW CLINICAL MODEL OF THE HADEN-HAUSSE<sup>R</sup> HEMOGLOBINOMETER<sup>3</sup>

RUSSELL L. HADEN, M.D., CLEVELAND, OHIO

IN 1930<sup>1</sup> I described a new hemoglobinometer which was developed in collaboration with the manufacturers, C. A. Haussner and Son. I have shown that hemoglobin estimations with this instrument check closely with determinations made by the oxygen capacity and iron content methods.<sup>2</sup> Our favorable experience with the Haden-Hausser hemoglobinometer has been verified in many other laboratories. The one objection to the instrument has been that it is essentially a laboratory instrument by reason of its size and original cost.

To overcome the objections of size and expense, a clinical model was later described.<sup>2</sup> This instrument has not proved as satisfactory as was hoped since

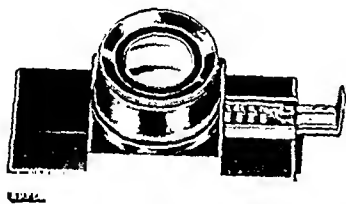


Fig. 1.

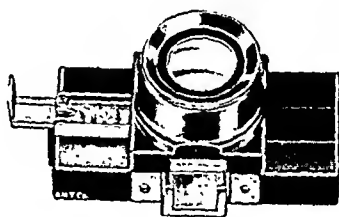


Fig. 2.

Fig. 1.—Front view of instrument, showing comparator slide in position.  
Fig. 2.—Rear view of instrument, showing light filter in position.

it is somewhat cumbersome to use, is fragile, and is not as sensitive as the laboratory model. Because of these difficulties, the model described herewith has been developed. It is essentially the laboratory model in a smaller size and without the light box. It is easily transported, it is inexpensive, and the readings are as accurate as those of the larger model.

The instrument is shown in Figs. 1 and 2, and in cross-section in Fig. 3. The molded Bakelite case holds the reading microscope, the movable comparator slide (Fig. 4) and the mirror for reflection of light from an outside source through the light filter. Filters for both daylight and artificial light are supplied. The comparator slide, except for the smaller dimensions, is exactly the same as the one used in the laboratory model and it is sealed in the metal carrier. The slide has a hemoglobin gram scale with a total range

\*From the Cleveland Clinic.

This instrument is made by C. A. Haussner and Son and is sold by Arthur H. Thomas Company, West Washington Square, Philadelphia.



from 7.5 to 18 gm. in the following intervals: 7.5, 8, 8.5, 9, 9.5, 10, 11, 12, 13, 14, 15, 16, 17, and 18 gm. This scale was arrived at by determining the oxygen combining capacity of many samples of blood throughout the range of the scale by use of the Van Slyke manometric blood gas method converted into hemoglobin content by the use of the Hüfner conversion factor of 1.34 ml. of oxygen per gram of hemoglobin. The glass used in the standard is the same as that shown by Razek<sup>3</sup> to have an absorption curve almost identical with acid hematin (Fig. 4). The comparator slide consists of a glass color standard of the same thickness throughout, which is in immediate juxtaposition to a wedge-shaped channel *E* for the dilution complement. This comparator is moved through the field of the reading microscope as the comparison is made. A series of illuminated rectangles, separated by alternate dark spaces,

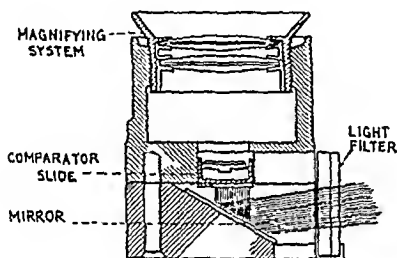


Fig. 3.



Fig. 4.

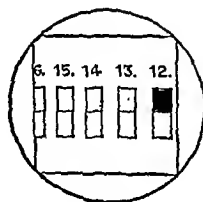


Fig. 5.

Fig. 3.—Cross-section view of instrument.

Fig. 4.—Comparator slide with cover glass in position.

Fig. 5.—Field of view in the new clinical model reading 14 grams.

then appears in the field of the microscope. The lower half of each rectangle constitutes the color standard complement, while the upper half of each rectangle constitutes the dilution complement when the wedge-shaped chamber is filled with diluted blood. The depths of the dilution complements vary because of the wedge-shaped channel.

In making hemoglobin determinations, the blood is diluted 1:20—or 1:10 if the hemoglobin content is probably below 7.5 gm., i.e., approximately 50 per cent—in a Thoma white cell pipette with N/10 hydrochloric acid. This low dilution ratio results in greater accuracy than is possible with the higher dilutions required in some instruments. After conversion of the hemoglobin into acid hematin, the diluted blood is run into the wedge-shaped dilution channel of the comparator at the end of the cover glass. The channel fills by capillarity.

An approximate determination of the hemoglobin is made by moving the comparator holder to the left until the upper illuminated half, the dilution complement, of the rectangle observed in the center of the field closely matches the glass color standard complement below. The rectangle in which the complements most nearly match is best determined by making such an approximate selection and then contrasting the upper complement with the adjacent color to the left and again with the adjacent color to the right. With the correct rectangle in the center of the field, the dilution complement to the left will be of lighter tint and the dilution complement to the right of a darker tint, and the dilution and color standard complements of this central rectangle will closely match.

The field of view in the new clinical model set for a reading of 15 gm. is shown in Fig. 5.

I have made comparative readings with this new instrument and with the laboratory model with results as shown in Table I.

TABLE I

SPECIMEN NO.	NEW CLINICAL MODEL GM.	LABORATORY MODEL GM.
1	12	12
2	14	14
3	10	10
4	16	16
5	13	13
6	11	11

In calculating the hemoglobin in percentage, any one of the hemoglobin standards can be used. A reading of 15 gm. would be transposed into percentage as follows:

By Haden's standard <sup>1</sup>	$\frac{15.0 \times 100}{15.4}$	= 97%
By Wintrobe's standard <sup>2</sup>	$\frac{15.0 \times 100}{14.5}$	= 103%
By Osgood's standard <sup>3</sup> (for men)	$\frac{15.0 \times 100}{14.7}$	= 102%
By American Society of Clinical Pathologist's standard	$\frac{15.0 \times 100}{17.3}$	= 87%
By standard determined locally in example given below	$\frac{15.0 \times 100}{15.4}$	= 97%

Since the number of grams of hemoglobin per 100 c.c. calculated to a count of 5 million cells has been found to vary in different laboratories and localities, it is desirable that each laboratory determine locally the standard to be used. The determination is simply, and fairly accurately done by making careful erythrocyte counts and hemoglobin estimations in grams on ten healthy, normal adults, although it is preferable to have a larger number. From data obtained, the number of grams of hemoglobin per 100 c.c. per 5 million red cells is calculated as indicated in Table II, and this figure is taken as 100 per cent of normal.

TABLE II

PATIENT	RED CELL COUNT PER C.M.M.	HEMOGLOBIN PER 100 C.C.
	MILLIONS	GRAMS
1	5.02	15.5
2	4.00	12.6
3	4.65	14.9
4	5.50	16.8
5	4.78	15.0
6	4.57	14.2
7	4.25	13.5
8	5.31	16.5
9	5.80	16.9
10	4.92	15.1
Mean of 10	4.88	15.1

The hemoglobin (X) per 5 million cells is

$$5.00 \times \frac{15.1}{4.88} \text{ or } 15.4 \text{ gm.}$$

This figure is taken as 100 per cent for the laboratory in which the data are obtained and when so used should give a color index of  $1.00 \pm 0.10$  in normal adults. The standards suggested by Haden, Wintrobe, and Osgood have been determined by this method but a much larger number of determinations have been made to diminish the error of the mean.

#### SUMMARY

A simplified model of the Haden-Hausser hemoglobinometer is described which is inexpensive, accurate, and simple to use.

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## TENTH NORMAL HYDROCHLORIC ACID AS A DILUTING FLUID FOR COMBINED LEUCOCYTE AND HEMOGLOBIN DETERMINATIONS\*

C. A. PONS, M.D., ASBURY PARK, N. J., AND W. P. BELK, M.D., ARDMORE, PA.

FOR use in the Haden-Hausser hemoglobinometer a 1-20 dilution of blood in tenth normal hydrochloric acid is made in a white cell diluting pipette. An economy of time and apparatus would result if the white cells could be counted in the same suspension.

Using a single sample of well-mixed oxalated blood, ten white cell counts were made in duplicate with tenth normal hydrochloric acid and with 0.5 per cent acetic acid as diluents. When subjected to standard statistical analysis, no significant difference was found between the means of the two series. Other samples were counted in duplicate and in no instance was there a greater difference than in the first series. Variation in the distribution of cells within the counting chamber was found sufficient to account for the major portion of the difference between the two series.

Ten suspensions in hydrochloric acid recounted after standing at least two hours showed good keeping qualities of the diluent, the second series being 8.7 per cent higher than the first.

Fifty duplicate counts on clinical specimens with both diluents showed an average deviation of 6.09 per cent.

The method has been used routinely for six months with perfect satisfaction.

The acid hematin suspension produces a light brown background and the cells are a little smaller in hydrochloric acid, but this is not sufficient to interfere with the accuracy of the counts.

A Reider pipette is found to be preferable to the Thoma because of its larger volume and greater potential accuracy. An effort was made to develop a special pipette for this purpose. However, objections to a pipette requiring a large volume of blood forced us to abandon this idea. We are indebted to Mr. S. Trenner, of Arthur H. Thomas and Company, Philadelphia, for aid with this pipette.

### CONCLUSION

Tenth normal hydrochloric acid is a suitable diluent for making white cell counts. The same preparation may be used for this purpose and for hemoglobin determinations.

We were pleased to learn that Dr. Frederick Boerner, of Philadelphia, was developing this idea at the same time that we were, and that his conclusions are the same as ours. We have just learned also that Walker† used

\*From the Department of Pathology, Fitkin Memorial Hospital, Asbury Park, N. J. and the Monmouth Memorial Hospital, Long Branch, N. J.

Received for publication, September 15, 1934.

†Walker, M. A.: Tenth-Normal Hydrochloric Acid as Diluent for Counting Leukocytes After Infusion of Solution of Acacia, *Am. J. Clin. Path.* 2: 347, 1932.

tenth normal hydrochloric acid for white cell counts after acacia injections, but did not advocate its routine use.

We wish to thank Dr. H. M. Lufkin, Instructor in Mathematics, University of Pennsylvania, for the statistical analysis.

## THE PERCENTAGE OF HEMOGLOBIN COMPARED TO THE VOLUME OF ERYTHROCYTES\*

### THE IMPORTANCE OF THIS RELATION IN CORRECTING THE VAN ALLEN DETERMINATION OF THE VOLUME OF PLATELETS

KAARE K. NYGAARD, M.D., AND DORIS L. DUXBURY, B.A., ROCHESTER, MINN.

THOMSEN, in 1919, published a report on a direct method for performing platelet counts. This method was based on the observation that platelets are evenly suspended in a plasma oxalate solution for a certain length of time (up to four hours), whereas the erythrocytes and leucocytes during this time will settle, thus leaving a clear oxalate plasma solution from which a drop may be transferred to a regular chamber for counting. Because of the dilution of the blood with oxalate solution, and because of the volume of the settled erythrocytes and leucocytes which will influence the dilution, it is necessary to correct the platelet count, and this necessitates determination of the hematocrit value in each case.

Gram, in 1921, simplified the correction. In 611 cases of disease of all kinds he found that there was constant and striking parallelism between the percentage of hemoglobin and the volume of cells. By determining the percentage of hemoglobin he could, therefore, obtain a good idea of the corresponding volume of cells, and the correction of the platelet count consequently could be based on determination of the percentage of hemoglobin.

Van Allen in 1925, published a report on his method of determining the volume of platelets; his method was based on the same principle as that applied in Thomsen's method for counting platelets. Consequently, it is necessary to apply a correction to the volume of platelets, as well as to the count in Thomsen's method, since the correction value is different and depends on the dilutions of blood oxalate solution. We have computed the correction for the platelet volume for any value as determined by hematocrit and have found it to vary from 2 per cent in a case in which the hematocrit determination was 10 per cent, to 15 per cent in a case in which the hematocrit gave a value of 75 per cent.

Again, taking up Gram's idea of substituting the hematocrit determination for the corresponding hemoglobin value, we have sought to simplify this

\*From the Mayo Clinic.

Received for publication, August 16, 1934.

Work carried out under direction of the Committee on the Study of Thrombosis, of the Mayo Clinic, Rochester, Minnesota. Submitted for publication, August 14, 1934.

correction. Gram, however, has not given any specific data regarding the relation of hemoglobin to volume of cells. We were not able to find such correlation values in the literature. This work, therefore, was undertaken in the hope that it would be of some practical importance in correcting platelet counts, and probably also similar questions that should arise in the future. Dr. H. L. Dunn, gave authoritative advice throughout this study.

Rowntree and Brown with the assistance of Roth, made simultaneous observations of different phases of the blood in a large series of cases. With the authors' permission we have gathered from their monograph simultaneous determinations of hemoglobin and of hematocrit values. The value for hemoglobin in grams per 100 c.c. of blood was determined according to a method which in reality was a combination of the method of Haden and that of Osgood, using Haden's standard and Osgood's technique.

The principle is the addition of dilute hydrochloric acid to whole blood, whereby oxyhemoglobin is converted to acid hematin. Comparison is undertaken with a standard of acid hematin in a colorimeter.

The volume of erythrocytes was determined by adding 5 c.c. of blood to 1 c.c. of a 1.6 per cent solution of sodium oxalate in an accurately graduated centrifuge tube and by centrifuging for thirty minutes at 3,000 revolutions per minute. "The volume of the corpuscles is expressed as a percentage of the volume of the sample of blood drawn."

TABLE I  
OBSERVATIONS MADE BY ROWNTREE AND BROWN WITH THE ASSISTANCE OF ROTH

	CASES	OBSERVATIONS
Normal men of varying body build	48	48
Normal women of varying body build	25	25
Normal subjects, whose blood volume and blood plasma fluctuated	22	44
Obesity	26	26
Chronic secondary anemia	15	15
Pernicious anemia	9	9
Polycythemia vera	49	49
Myelogenous leucemia	10	11
Lymphatic leucemia	4	4
Splenomegaly without anemia	6	6
Cirrhosis of the liver with splenomegaly	9	9
Hemolytic jaundice	11	11
Primary splenomegaly with anemia	18	18
Glomerulonephritis with edema	12	12
Subacute glomerulonephritis with developing anemia and incomplete diuresis	1	9
Nephrosis in the stage of edema	9	9
Cardiac edema	11	11
Diabetes with edema	3	6
Essential hypertension	10	10
Raynaud's disease	8	11
Thromboangiitis obliterans	5	5
Arteriosclerotic disease with occlusion	3	3
Arteriovenous fistula	7	7
Myxedema	8	8
Hyperthyroidism	4	4
Addison's disease	12	18
Diabetes insipidus	4	5
Diabetes mellitus	7	7
Chronic bronchitis, emphysema, and asthma	12	12
Total		412

The number of observations made by Rowntree and Brown (and Roth) and the type of cases they employed are given in Table I.

The distribution of the reading of grams of hemoglobin per 100 c.c. of blood, and the hematocrit value, are included in Table II.

The mean value for hemoglobin was 15.131 gm. The standard deviation was  $\pm 4.152$  gm. The mean hematocrit value was 41.966 per cent, and the standard deviation,  $\pm 10.455$  per cent. If  $x$  represents grams of hemoglobin per 100 c.c. of blood, and if  $y$  represents the hematocrit value in per cent, the coefficient of correlation,  $r$ , between  $x$  and  $y$  was found to be 0.885.

This is a very high correlation coefficient, indicating close correlation between grams of hemoglobin per 100 c.c. blood and the hematocrit value, thus substantiating Gram's statement. With this correlation definitely worked out, it was desired statistically to compute from any given value for grams of hemoglobin per 100 c.c. of blood, the correlated hematocrit value. The linear equation for this can be easily obtained from the constants already given and was found to be  $y = 2.2136x \div 8.47$ .

From any known value for grams of hemoglobin per 100 c.c. of blood  $x$  the correlated hematocrit value  $y$  can, therefore, be computed, according to the formula. Table II gives the result of this computation.

It will be easily seen that the correction values of Van Allen's platelet volume, computed on the basis of hematocrit values, can now readily be based on the corresponding number of grams of hemoglobin, thus obviating the hematocrit determination.

Aside from this practical point, the computation has revealed a theoretic problem of some importance. The high correlation coefficient found between grams of hemoglobin per 100 c.c. of blood and the hematocrit value, indicates that in the material concerned there is a constant amount of hemoglobin per volume unit of erythrocytes. This is another way of expressing what is termed the hemoglobin saturation of erythrocytes. Naegeli has pointed out that this hemoglobin saturation is maintained with surprising consistency, and is found to be a maximal one in normal cases. In many cases of severe anemia (chlorosis) this saturation is found to be decreased. In pernicious anemia there is supposed to be a maximal saturation. The material we have used does not include any cases of chlorosis; but does include a considerable group of anemias of different kinds. That the correlation coefficient is found as high as 0.86— seems to indicate that the hemoglobin saturation unit of erythrocytes in this material is maintained with rather persistence. We find, however, that for further information, the study here applied ought to be applied to larger and separate different kinds of diseases.

TABLE II  
RESULTS OF COMPUTATION

HEMO- GLOBIN, GM.	HEMATO- CRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT	HEMO- GLOBIN, GM.	HEMATO- CRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT	HEMO- GLOBIN, GM.	HEMATO- CRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT
		3.0	9.0	28.4		15.0	41.7	
			9.1	28.6		15.1	41.9	
			9.2	28.8		15.2	42.1	8.5
			9.3	29.1		15.3	42.3	
			9.4	29.3		15.4	42.6	
			9.5	29.5		15.5	42.8	
			9.6	29.7		15.6	43.0	
			9.7	29.9	6.0	15.7	43.2	
			9.8	30.2		15.8	43.4	
			9.9	30.4		15.9	43.7	
4.0	17.3		10.0	30.6		16.0	43.9	
4.1	17.5		10.1	30.8		16.1	44.1	
4.2	17.8		10.2	31.1		16.2	44.3	
4.3	18.0	3.6	10.3	31.3		16.3	44.5	
4.4	18.2		10.4	31.5		16.4	44.8	
4.5	18.4		10.5	31.7		16.5	45.0	9.0
4.6	18.7		10.6	31.9	6.5	16.6	45.2	
4.7	18.9		10.7	32.2		16.7	45.4	
4.8	19.1		10.8	32.4		16.8	45.7	
4.9	19.3		10.9	32.6		16.9	45.9	
5.0	19.5		11.0	32.8		17.0	46.1	9.2
5.1	19.8		11.1	33.0		17.1	46.3	
5.2	20.0	4.0	11.2	33.3		17.2	46.5	
5.3	20.2		11.3	33.5		17.3	46.8	
5.4	20.4		11.4	33.7		17.4	47.0	
5.5	20.6		11.5	33.9		17.5	47.2	
5.6	20.9		11.6	34.1		17.6	47.4	
5.7	21.1		11.7	34.4		17.7	47.7	
5.8	21.3		11.8	34.6		17.8	47.9	
5.9	21.5		11.9	34.8		17.9	48.1	9.6
6.0	21.7		12.0	35.0	7.0	18.0	48.3	
6.1	22.0	4.5	12.1	35.2		18.1	48.5	
6.2	22.2		12.2	35.5		18.2	48.8	
6.3	22.4		12.3	35.7		18.3	49.0	
6.4	22.6		12.4	35.9		18.4	49.2	
6.5	22.9		12.5	36.1	7.2	18.5	49.4	
6.6	23.1		12.6	36.4		18.6	49.6	
6.7	23.3		12.7	36.6		18.7	49.9	
6.8	23.5		12.8	36.8		18.8	50.1	
6.9	23.7		12.9	37.0		18.9	50.3	
7.0	24.0		13.0	37.3		19.0	50.5	
7.1	24.2		13.1	37.5		19.1	50.7	
7.2	24.4		13.2	37.7		19.2	51.0	
7.3	24.6		13.3	37.9		19.3	51.2	
7.4	24.9		13.4	38.1	7.6	19.4	51.4	
7.5	25.1	5.0	13.5	38.3		19.5	51.6	
7.6	25.3		13.6	38.6		19.6	51.9	
7.7	25.5		13.7	38.8		19.7	52.1	
7.8	25.7		13.8	39.0		19.8	52.3	
7.9	26.0	5.2	13.9	39.2		19.9	52.5	
8.0	26.2		14.0	39.5		20.0	52.7	
8.1	26.4		14.1	39.7		20.1	52.9	
8.2	26.6		14.2	39.9		20.2	53.2	
8.3	26.8		14.3	40.1	8.0	20.3	53.4	
8.4	27.1		14.4	40.3		20.4	53.6	
8.5	27.3		14.5	40.6		20.5	53.9	
8.6	27.5		14.6	40.8		20.6	54.1	
8.7	27.7		14.7	41.0		20.7	54.3	
8.8	27.9	5.6	14.8	41.2		20.8	54.5	
8.9	28.2		14.9	41.4		20.9	54.7	



TABLE II—CONT'D

HEMO- GLOBIN, GM.	HEMATO- CRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT	HEMO- GLOBIN, GM.	HEMATO- CRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT	HEMO- GLOBIN, GM.	HEMATO- CRIT, PER CENT	REDUCTION PLATELET VOLUME, PER CENT
21.0	55.0	11.0	24.2	62.0	13.0	27.4	69.1	14.0
21.1	55.2		24.3	62.3		27.5	69.3	
21.2	55.4		24.4	62.5		27.6	69.6	
21.3	55.6		24.5	62.7		27.7	69.8	
21.4	55.8		24.6	62.9		27.8	70.0	
21.5	56.1		24.7	63.1		27.9	70.2	
21.6	56.3		24.8	63.4		28.0	70.5	
21.7	56.5		24.9	63.6		28.1	70.7	
21.8	56.7		25.0	63.8		28.2	70.9	
21.9	56.9		25.1	64.0		28.3	71.1	
22.0	57.2		25.2	64.3		28.4	71.3	
22.1	57.4		25.3	64.5		28.5	71.6	
22.2	57.6		25.4	64.7		28.6	71.8	
22.3	57.8		25.5	64.9		28.7	72.0	
22.4	58.1		25.6	65.1		28.8	72.2	
22.5	58.3		25.7	65.4		28.9	72.4	
22.6	58.5		25.8	65.6		29.0	72.7	
22.7	58.7		25.9	65.8		29.1	72.9	
22.8	58.9		26.0	66.0		29.2	73.1	
22.9	59.2		26.1	66.2		29.3	73.3	
23.0	59.4		26.2	66.5		29.4	73.5	
23.1	59.6		26.3	66.7		29.5	73.8	
23.2	59.8	12.0	26.4	66.9		29.6	74.0	15.0
23.3	60.1		26.5	67.1		29.7	74.2	
23.4	60.3		26.6	67.3		29.8	74.4	
23.5	60.5		26.7	67.6		29.9	74.7	
23.6	60.7		26.8	67.8		30.0	74.9	
23.7	60.9		26.9	68.0				
23.8	61.1		27.0	68.2				
23.9	61.4		27.1	68.5				
24.0	61.6		27.2	68.7				
24.1	61.8		27.3	68.9				

TABLE III

HEMOGLOBIN AND HEMATOCRIT READINGS (ROWNTREE AND BROWN WITH ASSISTANCE OF ROTH)

HEMATOCRIT, PER CENT	HEMOGLOBIN PER 100 C.C. OF BLOOD, GM.*													
	0 to 1.9	2 to 3.9	4 to 5.9	6 to 7.9	8 to 9.9	10 to 11.9	12 to 13.9	14 to 15.9	16 to 17.9	18 to 19.9	20 to 21.9	22 to 23.9	24 to 25.9	26 to 27.9
10 to 14			1	1			1							
15 to 19				1	2									
20 to 24			1	6	6	4	1							
25 to 29				4	10	11	4							
30 to 34				1	4	10	17	15	1					
35 to 39					2	10	27	38	11	2				
40 to 44						12	13	62	40	14				
45 to 49				1			3	11	15	6	1			
50 to 54								1		2	4			
55 to 59								1		2			1	
60 to 64								1		3			1	
65 to 69								1			2		2	
70 to 74										1	6		4	1
75 to 79											2		2	1

\*Quantities in body of table represent cases.

Applying Karl Pearson's product-moment method of computing correlations, it has been possible to compute from any known value of grams of hemoglobin per 100 c.c. of blood, the correlated hematocrit value. The correlation is published in the form of a table. The practical applicability of such a table is clear when information is sought about two values and when determination of only one of the correlated values concerned is performed. An example is given in applying the table to the correction of the van Allen determination of platelet volume. The hemoglobin saturation of the volume unit of the erythrocytes is briefly discussed and in this series of 412 determinations is assumed to be remarkably constant.

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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**DEXTROSE**, Effects of Intravenous Administrations of Hypertonic Solutions of, Masserman, J. H. J. A. M. A. 192: 2084, 1934.

The effects of the intravenous injection of solutions of dextrose in various amounts and concentrations were studied in 85 normal patients. The administration of 50 gm. or less in 20 per cent solution produced no untoward clinical sequelae other than diuresis; however, the intravenous injection of 100 gm. or more in 35 to 50 per cent solution caused headaches and other adverse symptoms in 72 per cent of cases, whereas 58 per cent of the patients receiving 185 gm. or more suffered transient pyrexia. The intravenous administration of isotonic solutions caused a transient increase in cerebrospinal fluid pressure; with hypertonic solutions in effective concentration (100 to 200 gm. in 20 to 35 per cent solution) this initial rise was followed by a secondary fall in cerebrospinal fluid pressure, which in turn was superseded within an average of three hours by a tertiary increase to levels from 8 to 148 mm. of water above normal. The latter phenomenon is of clinical significance in relation to the late adverse effects sometimes observed in cases of intracranial hypertension treated by the intravenous injection of strongly hypertonic solutions.

**BILIRUBIN**, Ernst-Foster Method of Measuring in the Blood, Gajdos, A. Rev. Med.-Chir. des Malad. du Foie 9: 45, 1934.

Gajdos believes the Ernst-Foster method superior to Van den Bergh's in the determination of bilirubinemia, especially when the concentration in the blood is low.

The method follows:

1. To 1 c.c. of serum or plasma add 2 c.c. of acetone.
2. Mix well, filter, and centrifuge the filtrate.
3. Compare the supernatant fluid either in a colorimeter with a 1:6,000 dilution of potassium bichromate (representing 0.329 mg. of bilirubin per 100 c.c.) or against a series of standards prepared by graded dilutions.
4. Multiply the reading by 3.

If the bilirubin concentration is expected to be low only 1.5 c.c. of acetone is added to the serum, the reading then being multiplied by 2.5.

**CYCLOMASTOPATHY**: A Physio-Pathological Conception of Some Benign Breast Tumors, With an Analysis of Four Hundred Cases, Oliver, E. R., and Major, E. C. Am. J. Cancer 31: 1, 1934.

In this review the development of the breast has been considered, together with the histologic changes accompanying the initiation, course, and completion of the various cycles to which the organ is subject. A number of opportunities for abnormalities in behavior of the involved tissues have been demonstrated.

The dependence of the breast gland upon a complicated hormonal stimulation has been proved by work, the literature of which has been reviewed.

The historical aspects of benign tumors of the breast have been considered together with the development of an involved terminology.

The term "cyclomastopathy" is offered as a designation for the entire group of breast affections which present excessive connective tissue or epithelial proliferation, or both, in response to growth stimuli or as a manifestation of abnormal involution following normal response.

The term "cycylomastoma" is offered as a designation for localized areas of cycylomastopathy which give rise to palpable masses or to symptoms. The group of cases included in this study are examples of cycylomastoma.

Four hundred cases of benign breast lesions have been analyzed.

While not admitting of definite proof, it is the author's impression that cycylomastoma occurs more frequently in white than in colored women.

The peak of age incidence for all cases is reached between the twentieth and twenty-fifth years. The peak of incidence for white women is reached in the same age period; for colored women, in the period from fifteen to twenty years. The peak of incidence for both "fibro-adenoma" and "intraacinar myxoma" groups is reached in the same five-year period, i.e., between twenty and twenty-five. Consideration of the curves representing age incidence in the two groups reveals no significant difference. The group of "cystic adenoma" cases is too small to permit comparison.

No conclusion as to the influence of marital status upon the incidence of cycylomastoma can be drawn. In the married group, women who have borne children, that is, whose breasts have undergone lactation hypertrophy, outnumber those without children, two to one.

Although no portion of the breast is exempt, cycylomastoma has a predilection for the upper, outer quadrant. In the 236 cases with localization in one quadrant, 44.5 per cent of the lesions were in an upper outer quadrant.

A lump in the breast is the outstanding symptom, occurring in 83 per cent of cases. Pain occurred in 35 per cent and pain appearing or becoming intensified at the time of the menstrual period occurred in 15 per cent.

The average duration for all cases was thirty-six months; for the "fibro-adenoma" group, forty-four months; for the "intraacinar myxoma" group, thirty-one months. There was no significant racial difference.

Movability of the mass was described in only 56 per cent of all cases, a figure which does not indicate the actual general occurrence of this characteristic.

In 15.75 per cent of the total number of cases the masses were multiple, involving one or both breasts. In 5.7 per cent of cases the mass occurred in a generally lumpy breast.

Dimpling of the skin was observed in 11 cases and retraction of the nipple in 9 cases. In those cases in which a preoperative diagnosis was made, the lesion was considered benign in 94 per cent.

The operation of choice was simple excision with a zone of surrounding breast. Where the size of the mass demanded or where the diagnosis of malignancy or possible malignancy was made, amputation or the complete operation was done (12 per cent).

One hundred and ninety-eight cases have been followed for more than one year after operation, and the results are tabulated.

The stroma of the breast consists essentially of two types, the interlobular which includes the interlobar, and the intralobular which includes the periductal and periacinar.

The importance of the changes occurring in the breast at puberty in connection with the production of cycylomastoma and the possibility that many cycylomastomata discovered late in life date from the time of puberty, have been emphasized.

It has been demonstrated that hypertrophy of breast tissue may be grossly unilateral or bilateral, may be diffuse or isolated and, if isolated, single or multiple in one or both breasts; and may consist of epithelial or connective tissue hyperplasia or any combination of the two. Also, it has been demonstrated that connective tissue hyperplasia may be interlobular or intralobular or both.

Encapsulation has been found to be a mechanical phenomenon, without significant bearing upon the pathology of cycylomastoma. It is the author's belief that encapsulation is never entirely complete and that through their ducts the affected areas maintain connection with the remainder of the gland.

The general absence of uniformity of microscopic appearance throughout the affected areas has been emphasized.

The microscopic picture generally associated with the term "intracanalicular" may occur as a result of the involvement of an entire lobule as well. This type of pathology is, therefore, not necessarily intraductal.

Efforts to find a constant association between any particular type or consistency of connective tissue or any type of epithelial hyperplasia and rapidity of growth, duration of the mass, and age of the patient have been fruitless.

Epithelial cells lining ducts and acini with a tendency toward dilatation are likely to assume columnar form and acidophilic staining properties, and are not necessarily related to sweat glands or residual lactation.

In three cases fully differentiated squamous-cell epithelium and transitional stages between squamous and glandular types were found within areas of cecylomastoma in which proximity to the skin could be definitely eliminated.

The cases of "fibro-adenoma," "intracanalicular myxoma," and "cystic adenoma" have been incorporated into one group in the absence of sufficient differentiating features, either pathological or clinical, to justify their separation.

It has been found that all the microscopic appearances commonly associated with chronic cystic mastitis, single and multiple cysts, papillary cystadenoma or Schimmelbusch's disease, dilatation of ducts and acini, desquamation, and the assumption of columnar form and acidophilic staining, are encountered within these benign areas of cecylomastoma.

The evidence of response on the part of breast epithelium and connective tissue to hormonal stimuli, in the elaboration of which the ovary and anterior pituitary body at least must play a part, has been accepted. The fact that such hormonal control introduces a variable factor and a potential pathogenic agent has been stressed.

A second variable factor and potential pathogenic agent has been postulated, namely, a lack of uniformity of tissue behavior in response to stimulation. Evidence of such a lack of uniformity, even in processes considered normal, has been adduced.

It has been pointed out that every evolutionary or hyperplastic phase in any of the breast cycles is followed normally by an involutionary phase, which in pregnancy and menstruation has its own proliferative aspects. It immediately follows that involution offers its own peculiar opportunities for abnormal behavior.

Any relationship between cecylomastoma and carcinoma has been denied. There may be a slight tendency on the part of these masses to undergo sarcomatous change.

#### **BLOOD SEDIMENTATION, The Significance of Repeated Red Cell Sedimentation Rate Determinations in Pulmonary Tuberculosis, Siltzbach, L. E. Am. Rev. Tuberc. 29: 673, 1934.**

The E.S.R. curves (Cutler) were studied on 494 patients with pulmonary tuberculosis: 1170 individual tests were performed. The data so obtained were analyzed, and correlative studies with the constitutional and local manifestations of the disease were made. It was found that curves reflecting moderate and severe activity occur with greater frequency in patients with fever, rapid pulse, loss of weight, positive sputum, cavity-formation, extensive lesions, and extrapulmonary tuberculosis. The E.S.R. curve reflects the progress of the disease and a change in E.S.R. curve often precedes the change in clinical symptoms and signs. It is of value, therefore, in prognosis in pulmonary tuberculosis. In collapse therapy the E.S.R. may be utilized as a gauge of the effectiveness of the treatment. The use of E.S.R. curve determinations in routine sanatorium and private practice in tuberculosis is recommended.

#### **MENINGITIS TUBERCULOUS, The Tryptophane Test in, Rosenblatt, M. B. Am. Rev. Tuberc. 29: 668, 1934.**

The presence of a positive tryptophane test is strongly suggestive, but not pathognomonic, of tuberculous meningitis.

Hemorrhagic, purulent, and xanthochromic fluids give "false positive" reactions which are usually distinguishable from those obtained in tuberculous meningitis by the

purplish color. Indistinguishable positive reactions may be obtained with clear spinal fluids in which the protein is increased.

There is sufficient evidence to warrant the use of the tryptophane test as a routine procedure in the examination of the spinal fluid.

**PREGNANCY, Studies in Pernicious Anemia of: Tropical Macrocytic Anemia as a Deficiency Disease With Special Reference to the Vitamin B Complex, Wells, L. Indian J. M. Res. 31: 669, 1934.**

Evidence is adduced and discussed that tropical macrocytic anemia is a simple condition of dietary deficiency.

The curative action of marmite (a yeast extract) in this condition already reported has been confirmed.

The nature of the hemopoietic factor was investigated by the use of various specially prepared fractions of the vitamin B complex.

By this means the recognized constituents of the vitamin B complex were excluded.

The hemopoietic fraction of marmite was found to be water-soluble and heat-stable in acid medium and not to be precipitated or inactivated by 80 per cent alcohol.

**TUBERCULIN REACTION, Quantitative Study of, in Childhood Tuberculosis, Johnston, J. A., Howard, P. J., and Maroney, J. Am. Rev. Tuberc. 29: 652, 1934.**

The reaction to tuberculin in the childhood type of tuberculosis has been studied throughout the evolution of the primary complex and found to follow a curve which rose to a high peak following the absorption of the parenchymal lesion and corresponding with the maximal involvement of the hilar lymph nodes, and then to decline with the diminution in size of these nodes, and their calcification. While this course of events is usually paralleled by peripheral blood changes associated with healing, the same tuberculin course was seen when x-rays showed absorption but the blood picture remained equivocal, or even showed retrogression. It is the authors' feeling that these data favor the concept that the allergic and immune phenomena, in so far as the latter are reflected in the peripheral blood, are relatively independent.

**TRICHOMONAS VAGINALIS, Incidence and Treatment of, in Pregnancy, Glassman, O. J. A. M. A. 102: 1748, 1934.**

Vaginal discharge during pregnancy is often dismissed as a normal physiologic change but is frequently associated with *Trichomonas vaginalis*.

In a series of 309 pregnant patients personally examined, *Trichomonas vaginalis* was found in 20.7 per cent.

The symptoms are essentially the same as in the nonpregnant, and the diagnosis is easily made by a hanging drop.

The cases reported showed no increased puerperal morbidity.

The multiplicity of treatments indicates that a satisfactory method is not available, but a crystalline phenol powder has been used with good effect in this series.

Some cases are apparently cured spontaneously during labor and the puerperium.

The life cycle of the trichomonas requires further study, but in the meantime many patients may be given much relief from the annoying symptoms and leucorrheal discharge associated with this organism by more careful attention to the microscopic examination of the vaginal secretion.

**B. DIPHTHERIAE, Differentiation of, Dockeray, G. C. Irish. J. M. Sc. 97: 12, 1934.**

The medium following is simple and easy to prepare and has been found of value in the isolation of *B. diphtheriae*.

Two Erlenmeyer flasks of about 500 c.c. capacity are taken and 150 c.c. of 5 per cent agar is put in one. This is sterilized in the autoclave, and while still liquid is used to prepare the medium. In the other flask is 150 c.c. of tryptic digest broth. To the broth

from 20 to 30 c.c. of sterile citrated human blood is added, followed by 120 mg. of potassium tellurite dissolved in a few cubic centimeters of sterile saline solution. The tellurite-blood-broth mixture is warmed to about 40° C. and is then poured into the melted agar, which has been allowed to cool to about 70° C. The mixture is heated in a water bath at 75° C. for ten minutes and, when cooled to from 50 to 55° C., is poured as plates.

**INTRAVENOUS THERAPY, Study of Hyperpyrexia Reaction Following, Banks, H. M.**  
*Am. J. Clin. Path.* 4: 260, 1934.

The reaction that follows intravenous administration of solutions is an entity characterized by an immediate and prompt rise of temperature in extremely high degree and usually associated with a severe rigor lasting about twenty to thirty minutes. This clinical picture is not to be associated with shock and is entirely separate and distinct from it. Intravenous reaction has a definite, specific, etiologic factor. This factor is the introduction of dead or living products of bacterial growth or cultures of, *Pseudomonas seissa* or ureae, into the blood stream of the individual.

These organisms were isolated in pure culture from the offending solution, reinjected into the individual and produced the same clinical picture. It was not possible to recover the organism from the blood stream of the individual suffering from a reaction, even though cultures were made at various times during the rigor.

The pyrogenic substance may be used as a therapeutic agent to produce hyperpyrexia.

**TYROSINURIA, Origin and Significance of, in Disease of the Liver. Lichtmann, S. S.**  
*Arch. Int. Med.* 53: 680, 1934.

Tyrosinuria was studied in health and in disease by the tyrosinase method. Its occurrence and estimation proved to be of significance in the diagnosis and prognosis of diseases of the liver and bile passages and in the interpretation of its mode of origin.

The quantitative observations in excretion of tyrosine confirm the view that the abnormal amounts of amino-acids excreted in the urine of patients with acute atrophy of the liver originate mainly from autolyzed liver tissue. While it is conceded that in acute diffuse degeneration of the liver the function of the liver in deamination may fail and contribute to the amino-aciduria, this factor plays only a minor rôle. Studies of the amino-nitrogen content of the blood and urine in some cases of subacute atrophy of the liver with tyrosinuria indicates that in this less acute form of degeneration of the liver tyrosine may appear in the urine when there is no apparent disturbance in the metabolism of amino-acids.

Observations on the rate of excretion of tyrosine are of diagnostic significance. Continuous massive tyrosinuria occurs only in cases of acute yellow atrophy with a rapid and fulminant course. Transitory minimal and moderate tyrosinuria occurs in cases of subacute atrophy of the liver, in degenerating neoplasm of the liver, in toxic degeneration of the liver and uncommonly in obstructive jaundice of long standing due to stone. Inflammatory lesions of the bile passages do not of themselves give rise to tyrosinuria.

Extrahepatic foci of autolysis, such as degenerating tumors of the lung or extensive sloughs of the skin, may give rise to minimal or moderate amounts of tyrosine in the urine.

The transitory nature of minimal or moderate tyrosinuria is demonstrated by repeated tests. During the phase of recovery from degeneration of the liver, the products of parenchymal autolysis have already been absorbed, and tyrosine vanishes from the urine, but with a fresh attack of degeneration of the liver, tyrosine reappears in the urine. In the stage of repair, namely in coarse nodular cirrhosis, or in the terminal stages of subacute yellow atrophy with a critical reduction in the functioning parenchyma of the liver, tyrosine may not be demonstrable in the urine.

Tyrosinuria may occur without jaundice either in cases of extrahepatic origin or in those of demonstrable disease of the liver.

Quantitative observations with respect to tyrosine are of prognostic value. In cases of minimal tyrosinuria the patients are more likely to recover, whereas cases of massive tyrosinuria terminate fatally in a short time. Mounting tyrosinuria indicates rapidly progressive degeneration of the liver. However, the absence of tyrosinuria in cases of degeneration of the liver does not warrant an optimistic prognosis.

**CHOLESTEROL, Blood, and Thyroid Disease, Hurxthal, L. M.** Arch. Int. Med. 53: 762, 1934.

Postoperative myxedema is accompanied by hypercholesteremia.

Subtotal thyroidectomy may be followed by hypercholesteremia without clinical myxedema. This is interpreted as a transient thyroid deficiency.

Subtotal thyroidectomy may be followed by low metabolic rates without hypercholesteremia. In these cases myxedema is seldom found.

Röntgen therapy can produce transient hypercholesteremia with or without clinical myxedema.

Removal of part of a thyroid gland affected by chronic thyroiditis may be followed by hypercholesteremia and myxedema.

Thyroid deficiency produces myxedema and hypercholesteremia, but at times myxedema may be clinically imperceptible.

Hypercholesteremia, when not explainable on any other basis, may be considered as possibly of thyroid origin, and is a rational indication for thyroid administration.

The finding of hypercholesteremia, in the absence of its few other common causes, points more specifically to thyroid deficiency than does the finding of a low metabolic rate. Finding both renders the possibility of thyroid deficiency extremely likely.

The relationship between the blood cholesterol and the basal metabolism is usually reciprocal when they undergo change as the result of variations in the activity of the thyroid gland or thyroid substance in the body.

The blood cholesterol provides another variable which may be used as a guide in the treatment of thyroid disease.

**IRON, Constancy of, in the Blood Plasma and Urine in Health and Disease, Marlow, A., and Taylor, F. H. L.** Arch. Int. Med. 53: 551, 1934.

The iron content of the blood plasma of normal persons varies between 0.4 and 0.7 mg. per hundred c.c., while that of the urine ranges from 0.03 to 0.8 mg. per twenty-four hours.

The iron content of the blood plasma and urine of three patients with hypochromic anemia due mainly to chronic loss of blood and of two patients with pernicious anemia soon after remission had been inaugurated was found to vary within normal limits. The oral administration of ferrie ammonium citrate to these patients and to normal persons caused no definite increase in the iron content of the blood plasma or of the urine. However, in the normal person studied there was perhaps a slight increase in the urinary excretion of iron following the oral administration of this substance in an acid-buffered medium.



## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

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### A Synopsis of Medicine\*

WHILE it is not the purpose of a "synopsis" to serve as a textbook of medicine, as a source of ready reference and a means of review, such works have an eminently practical and valuable place in the practitioner's library.

Tidy's is among the best known of such works so that it is not surprising to see a new sixth edition which has been extensively revised to keep abreast of the new and important advances which have been made since the last edition in 1930.

The revision has been thorough and extensive, so much so that the book has been entirely reset, the new material making an addition of about twenty-five pages.

Among the sections in which many changes have been made are those on Deficiency Diseases, Diseases of the Parathyroid Glands, and Diseases of the Bones.

Nineteen new articles have been added and extensive changes made throughout the text in general.

The book can be recommended confidently to the practitioner as reflecting the status praesens of the entire field of medical practice.

### Tumors of the Female Pelvic Organs†

IN THIS book Dr. Meigs presents a survey of the present knowledge of the various tumors, both benign and malignant, that occur in the female pelvic organs.

While the material is based chiefly upon a series of cases from the Massachusetts General Hospital, these have been so carefully studied and verified and the pertinent literature so thoroughly reviewed, that the book may justly be regarded as an extremely valuable and authoritative presentation of the subject.

In view of the extensive and almost bewildering accumulation of literature in this field, this book should be heartily welcomed by all concerned with it, as who should not be.

The plan upon which the volume is constructed is eminently practical. Beginning in each instance with a survey of the present pathologic knowledge of the tumors in each organ, Dr. Meigs then discusses in correlation with these facts the clinical signs and symptoms and finally the appropriate methods of treatment and their results.

The style is clear and simple and the volume bears the stamp, not only of a thorough acquaintance with the literature, marked by discriminating analysis, but also of an extensive and well-evaluated practical experience.

Both the author and the publishers are to be congratulated on the excellent illustrations.

To the pathologist, the surgeon, the roentgenologist and the practitioner Dr. Meigs's book should come as a standard reference text not likely to be displaced for some time to come.

The book may be highly commended as a contribution of great practical value and importance.

\*A Synopsis of Medicine. By Henry Letheby Tidy, Physician to St. Thomas' Hospital, London, etc. Ed. 6, cloth, pp. 1112. Wm. Wood & Co., Baltimore.

†Tumors of the Female Pelvic Organs. By Joe Vincent Meigs, M.D., Instructor in Surgery, Harvard Medical School, with a foreword by Robert B. Greenough, M.D., President-elect, American College of Surgeons, 1933-34. Cloth, pp. 533, 261 illustrations. The Macmillan Company, New York.

### Physical and Clinical Diagnosis\*

**T**HAT this book has achieved twenty-four German and two English editions will not be surprising to the reader for in small compass it covers, and covers clearly and well, the entire field of physical and clinical diagnosis.

Intended to serve as a succinct and compact compendium for use as a handbook of ready reference, it achieves its purpose excellently well. While succinct it is not laconic and comprises a wealth of material of practical value. That so much can be contained in so little space is largely due to the format, the volume being pocket size. Unlike so many "pocket manuals" the type is of comfortably readable size and character.

This book may be highly recommended.

### Die operative Technik des Tierexperimentes†

**T**HE second edition of this manual which comprises the fifth division, Part 3 c of Abderhalden's "Handbuch der biologischen Arbeitsmethoden" will be found of inestimable value for those who are engaged in the laboratory sciences. The author describes the habits and care of the ordinary laboratory animals, the various anesthetics and their mode of administration, and the question of asepsis quite thoroughly. Detailed descriptions are given of the various operative procedures on almost every organ of the body. The text is replete with excellent illustrations.

H. B. HAAG.

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\*Physical and Clinical Diagnosis. By Dr. Otto Seifert, late Professor of Medicine, Wuerzburg, and Dr. Friederich Muller, Professor of Medicine, II Clinic, Munich, Second English Edition. Authorized translation by E. Cowles Andrus, M.D., Associate Professor of Medicine, Johns Hopkins Hospital. Cloth, pp. 561, 152 figures, 4 color plates. J. B. Lippincott & Co., Philadelphia.

†Die operative Technik des Tierexperimentes. (The Operative Technic of Animal Experiments.) By H. F. O. Haberland, Dr. med., Professor of Surgery, University of Köln. 434 pages, with 382 illustrations. Urban & Schwarzenberg, Berlin, 1934.

# The Journal of Laboratory and Clinical Medicine

VOL. 20

APRIL, 1935

No. 7

Editor: WARREN T. VAUGHAN, M.D.

Richmond, Va.

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## EDITORIAL

### The Mills of the Gods and Anesthesia

THE mills of the gods grind slowly. And there are perhaps few fields of human endeavor in which this has been better illustrated than in that of anesthesia, for the long and despairing silence of more than two thousand years between the discovery of opium and the first practical use of ether, nitrous oxide and chloroform to relieve human suffering serves to illustrate in a singularly striking manner the weakness and the strength, the failures and the successes of the healing art. It is interesting to note that even so late as 1839 the great French surgeon Velpeau was able to write that "to escape pain during surgical operations is a chimera for which no solution need be expected within our time." This was only three years before Long used ether in a surgical operation. And it certainly behooves the medical profession to anxiously view the future in hopefulness that history may thus tend to repeat

itself with reference to some of the other great stumblingblocks that have baffled medical science for much more than two thousand years. But notwithstanding the agonies and horrors through which the human race passed before anesthesia got its start, it now seems certain that no fault can be found with the speed of advancement nor with the volume of knowledge by which anesthesia has been enriched in very recent years. Indeed it seems very probable that the specialty of anesthesia is now further advanced and has attained a more certain and satisfactory command of its particular problems than is the case with any other specialty in the whole field of medicine.

It has now been just twenty years since a paper on the invention of the closed system for anesthesia with absorption of carbon dioxide constituted the first article<sup>1</sup> which introduced this JOURNAL to the medical profession in October, 1915. In the meantime ethylene and cyclopropane have been added to the list of gaseous anesthetics. And vinesthene (divinyl ether) which has much in common with the gases, has been introduced. It now seems certain that hereafter a constantly increasing number of anesthetics produced by these anesthetic agents will be carried out by the use of the carbon dioxide absorption method. The increased safety (for these anesthetics are explosive and inflammable), the reduced cost and the greatly improved type of anesthesia made possible by this method will insure its eventual almost universal adoption.

Trichlorethylene,<sup>2</sup> which has general anesthetic properties and is neither explosive nor inflammable, may be so cheap that it can better be used without the closed system, but even here the relative advantages are yet to be investigated.

While none of these anesthetics are absolutely ideal still they represent a great stride forward. The introduction of the newer barbiturates and similar hypnotics as basal anesthetics, or in some instances, as the sole anesthetic agent used, also represents a distinct advancement in this field.

Cyclopropane, the newest gas to be used extensively as a general anesthetic, is a three carbon cyclic compound isomeric with propylene and having the formula  $C_3H_6$ . It was first prepared by Freund<sup>3</sup> in 1882, while Lucas and Henderson<sup>4</sup> in 1929 carried out a series of animal and chemical experiments and announced the general anesthetic properties of the gas on animals and also the observation that only 9 per cent of cyclopropane in oxygen would produce unconsciousness when inhaled by these workers. Ten to 20 per cent of the gas in oxygen produced satisfactory anesthesia, with but little change in the vital physiologic functions of the body. Waters and Schmidt as early as 1932 were working with the gas, and in 1934 Stiles, Neff, Rovenstine and Waters<sup>5</sup> published a most interesting report on four hundred and forty-seven anesthetics with cyclopropane. With the especial caution that the use of the gas should be regarded as still in the experimental stage for another year these workers reported satisfactory results in this series of anesthetics. The gas acts quickly and powerfully and complete relaxation can nearly always be obtained. Waters and his coworkers failed in only two of the four hun-

dred and forty-seven cases mentioned above. In one of these ether produced relaxation only after a huge amount had been administered. The gas is more rapidly flexible than ether and consequently the depth of anesthesia can be regulated much more quickly. Consciousness is usually regained in four or five minutes though the occasional patient may require as long as ten to twelve minutes. This recovery time corresponds well with that following trichlorethylene. Cyclopropane is apparently rapidly exhaled from the body and when the patient revives he thereafter remains awake. Postoperative complications compare favorably with those of other anesthetics in general use. It seems probable that in this respect trichlorethylene may be freer from postoperative nausea, etc., than cyclopropane or most other anesthetics.

Cyclopropane is explosive but less so than ethylene. There is also a difference in the relative explosiveness of various percentage mixtures of each of these gases with oxygen. A good anesthetic mixture of ethylene and oxygen will hardly explode although it may burn like illuminating gas. But a 10 or 15 per cent mixture of ethylene with 85 or 90 per cent of oxygen is very explosive. On the other hand it appears that the most explosive mixture of cyclopropane with oxygen is in the neighborhood of 50 per cent of each gas. The best anesthetic mixture of cyclopropane with oxygen, however, is within the limits of 10 to 20 per cent of the gas with oxygen. This mixture will hardly explode, but 25 per cent cyclopropane with 75 per cent oxygen is moderately explosive. From this it will be seen that the anesthesia is started with a mixture that is not explosive, and it is only as more and more of the gas is given to increase the depth of the anesthesia that a really explosive mixture is produced. Just the reverse of this happens in starting off an anesthesia with ethylene. Here one may hurry to increase the percentage of ethylene to get past the most dangerous mixture, while with cyclopropane one may hold back the gas to keep within safer limits.

Cyclopropane appears to be too powerful and too rapid in its action to render its use safe within the medical profession at large until a period of education in its action and administration can be carried out. This is especially true for those who have not had special training in the field of anesthesia. For the gas produces a rapidly moving series of symptoms which differ considerably in several respects from those produced by the other older anesthetic agents. Trichlorethylene<sup>6</sup> on the other hand acts more slowly, there is often a rather marked excitement stage and the patient does not seem to be so profoundly depressed. But the (*central*) analgesic properties of the drug, even in very small doses, are often well marked as may be seen in its use to relieve the pains of the *douloureux*,<sup>7</sup> in about 10 or 11 per cent of which patients may be permanently cured under its use.

The splendid investigations which are now being vigorously carried forward along these lines by such workers as Woodbridge, Waters, Hammond, Leake, Bourne, Herb, Lundy, Luekhardt, Rovenstine, Guedel, Brown, and many others can leave no doubt but that at last the mills of the gods are grinding rapidly as well as "exceeding fine" so far as anesthesia is concerned.

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—D. E. J.

# *The Journal of Laboratory and Clinical Medicine*

VOL. 20

MAY, 1935

No. 8

## *CLINICAL AND EXPERIMENTAL*

### BLOOD GROUPS, THEORY AND MEDICOLEGAL APPLICATION\*

PHILIP LEVINE, M.A., M.D., MADISON, WIS.

IN THE past few years much progress has been made in the study of blood groups and individual differences of human and animal blood. It is, therefore, essential for the practicing physician to acquaint himself thoroughly with the more recent advances in this field which have a bearing not only on the practice of medicine, but also with those aspects of the subject dealing with exclusion of paternity and examination of blood stains in criminal cases.

The first observations on the existence of individual differences of human blood were made as far back as 1900 by Landsteiner.<sup>1</sup> An accurate description of the blood groups and their bearing on the outcome of successful transfusion, was given by Landsteiner.<sup>†</sup>

#### THEORY OF THE FOUR BLOOD GROUPS

The scheme of the four blood groups, as is well known, results from the distribution of two agglutinable substances A and B in the cells, and two corresponding agglutinins in the serum. The arrangement of the groups can be readily verified in tests made with the serums of about 12 to 20 individuals on the corresponding cells. In such "cross tests," preferably made in small test tubes, the 4 blood groups are readily characterized by considering both the reactions of the red cell and also the corresponding isoagglutinins. Thus the Group 0 (I of Jansky, IV of Moss) is defined by a cell which does not react with any

\*From the Department of Pathology and Bacteriology, University of Wisconsin. Received for publication, October 22, 1934.

†In America the earliest references to blood groups and transfusion are found in papers by Hektorn (J.A.M.A. 48: 1739, 1907; J. Infect. Dis. 4: 297, 1907). Ottenberg mentions the use of compatibility test prior to transfusion in a paper in the Annals of Surgery 47: 486, 1908.

serum, and by the existence of agglutinins in the serum which agglutinate the cells of the 3 other types. This is the so-called universal donor, since its cell, by virtue of its inagglutinability can be injected intravenously, as a rule, into persons of any group with impunity. This type, which in the white race is found in about 40 to 50 per cent of all individuals, is to be contrasted with a much rarer type (3 per cent), Group AB, which cell is agglutinated by the serums of all other individuals and whose serum contains no agglutinins. Such individuals are the so-called universal recipients (Group IV Jansky, I Moss), since they will accept generally intravenous injections of any cells by virtue of lack of agglutinins. The two remaining types of blood (Groups A and B) are "cross-specific," since the serum of the one type will agglutinate the cell of the other and vice versa.

The two can be differentiated by their varying frequencies in the white population, the A type occurring more frequently (40 per cent for A, and 10 to 15 per cent for B).

The relationships of the four blood groups, as well as the various terminologies, are summarized in Table I. While the terminologies of Moss and Jansky are still being employed in many hospitals, the nomenclature by letter has the very decided advantage since the group is identified by the agglutinable property present in the red blood cell. It must be pointed out that the grouping by letters has been recommended by a special health committee of the League of Nations and also, by the American Association of Immunologists. In order to avoid confusion, and until hospitals agree to use the Landsteiner classification, physicians must acquaint themselves with the three nomenclatures.

TABLE I\*

AGGLUTININS IN SERUM OF GROUP			AGGLUTININ	RED BLOOD CELLS OF GROUP			
JANSKY	MOSS	LANDSTEINER		O	A	B	AB
I	IV	O	Anti-A	0	+	+	+
II	II	A	Anti-B	0	0	+	+
III	III	B	Anti-B	0	+	0	+
IV	I	AB	Anti-A	0	0	0	0

\*+ indicates agglutination.

0 indicates absence of agglutination.

The elements in the serum, the isoagglutinins, are generally identified by the Greek letters  $\alpha$  and  $\beta$  or the corresponding terms anti-A, and anti-B.

The proof for the theory of 2 agglutinable substances isoagglutinogens and two corresponding agglutinins was furnished many years ago by Landsteiner.<sup>3</sup> Accordingly blood cells of Group A remove isoagglutinin anti-A but not anti-B from serum 0; isoagglutinin anti-B is specifically absorbed by bloods containing isoagglutinin B; both antibodies are absorbed by blood cells of Group AB. (This technic is carried out by allowing one volume of serum to mix with one-half volume of packed and washed red blood cells for a short time and repeating the process if the absorption is incomplete. Tests for the completeness of the absorption are made by adding, in small tubes, one drop of the clear supernatant fluid to one drop of a 2½ per cent suspension of washed blood.)



As is well known the blood group of any individual can be readily determined by using as reagents serums of Groups A and B (Table I, lines 2 and 3). The technic of performing the tests is very simple, since the reactions, as a rule, are powerful, and withstand considerable dilution; they are stable over a range of temperature beyond 37° C. Nevertheless there are a number of phenomena which may cause confusion, particularly pseudoagglutination, cold agglutination, and the so-called atypical agglutination exhibited by about 3 per cent of all human serums.<sup>4, 5</sup>

Pseudoagglutination or rouleaux formation is due to the existence of a sticky substance in the serum of pregnant women or in patients suffering from certain infectious diseases, i.e., tuberculosis, pneumonia, and rheumatic fever. When observed in the test tube, the phenomenon is characterized by a rapid sedimentation of the cells; on examining such blood under the microscope, intense rouleaux formation is observed which in some cases may closely simulate typical isoagglutination. This form of agglutination does not withstand even mild dilution, and in order to avoid errors attributable to pseudoagglutination, it is usually sufficient to dilute the stock grouping serums in performing the test.

The difficulties incidental to cold agglutination are not so serious, because the cold agglutinins are rarely operative under the usual conditions of carrying out blood grouping tests, i.e., room temperature.

Errors in blood grouping undoubtedly have been made because the grouping serums employed had agglutinins other than anti-A or anti-B acting at room temperature on blood cells irrespective of the groups. A systematic investigation of serums containing these atypical agglutinins has established the following facts. Although the reactions are distinct at room temperature, they are, as a rule, much weaker than those of typical isoagglutination; with few exceptions, they disappear if the tests are allowed to remain at 37° C. These reactions do not apparently play any significant rôle in the selection of donors for transfusion or in posttransfusion reactions. The specificity of the most common atypical agglutinins are such that they define the so-called subgroups A<sup>1</sup> and A<sup>2</sup> (agglutinins anti-A<sup>1</sup> and anti-A<sup>2</sup>) and another factor called P. Brief mention of these factors will be made below.

In order to avoid these confusing reactions, serums containing atypical agglutinins should not be used as testing serums. Actually there is no difficulty in determining the group of such serums since the abnormality exists only in the serum, the corresponding red cell behaving in a normal manner. The occurrence of these atypical agglutinins and also the cold agglutinins is evidence for the assumption that there exists a great variety of individual blood differences, aside from the classical four blood groups.

The isoagglutinable properties A and B are not confined to the blood cells, but have been shown to be present in sperm cells<sup>6</sup> and in the cells of many organs such as liver, kidney, and lung, but not in the brain.<sup>7, 8</sup> This effect cannot be demonstrated directly by the agglutinin reaction on account of the difficulty of obtaining uniform suspension of organ cells, but rather by a capacity of these organ cells to absorb specifically isoagglutinins anti-A or anti-B. By means of specific inhibition of the isoagglutinin reaction, group specific substances have

been demonstrated in serum, urine, saliva, and seminal fluid. This specific reaction of soluble body fluids and isoantibodies is important from a theoretical point of view, since solutions are available to study the chemical properties of the group specific substances in these fluids, and some preliminary results by Schiff,<sup>9</sup> Freudenberg<sup>10</sup> and Landsteiner<sup>11</sup> indicate that not proteins, but carbohydrates are present in the active substance.\* Studies with urine or saliva are particularly convenient on account of the difficulty of extracting completely all the active material from red blood cells.

Brief mention will be made later of the medicolegal application of the facts stated above (see p. 794).

*The Factors A and B in Transfusion.*—The rôle of the blood groups in the successful outcome of transfusion was thoroughly established as a result of the ample experiences acquired during the Great War and confirmed in the period following the War. Large series of cases are available which show that transfusion with compatible blood carried out with any of the recognized methods, is a perfectly safe operation. On the other hand, except in those comparatively rare combinations of poorly agglutinable blood cells and weak isoagglutinins in the patient's serum, accidental transfusion of incompatible blood even in comparatively small quantities results in immediate violent reactions which are frequently fatal.†

Nowadays the selection of a compatible donor in the larger communities should be made much easier by organization of donor agencies, preferably in connection with a large university hospital, or the local medical society which lays down certain necessary and obvious requirements dealing with periodic examination of donors, frequency of donating blood, and price of blood.

Lists of donors of all 4 groups are readily available so that, knowing the group of the patient, all that is required is a selection from the list of a donor of the same group, followed by a suitable direct cross matching of blood of donor and patient. This additional procedure is essential, since it serves at the same time also as a control of the blood grouping test made with the stock grouping serums. This order of the procedures, namely, first a blood grouping and then a direct matching, is preferable, otherwise it may be necessary to call several prospective donors before one is found whose blood is compatible as shown by the direct matching.

There has been much discussion both for and against the use of Group O individuals as universal donors for patients of all groups. Undoubtedly rare Group O individuals exist with unusually active isoagglutinins anti-A or anti-B or both (dangerous universal donors<sup>13, 14</sup>), and while there is no reason to believe that injection of the previously washed inagglutinable blood O cells into any individual will be harmful, clinical experience supports the view that the injection of active agglutinins may not be entirely harmless. In a well-organized donor agency, not only should the blood cells be studied, but also the potency of the agglutinins ought to be determined. Thus, a list of the so-called dangerous

\*See earlier work of Landsteiner, K., and Levene, P. A.: *J. Immunol.* 14: 81, 1927.

†For literature see Levine.<sup>15</sup>

donors will be established, and only those Group O donors with a low titer of agglutinins can be used, if necessary, for patients in other groups, particularly for the rarer Groups B and AB. In a similar manner Group A and B donors with comparatively weak agglutinins anti-B and anti-A, respectively, should be selected who may also be used, along with suitable Group O donors, as donors for patients in Group AB (universal recipient). With this information on hand, a donor can be immediately employed with a very great probability of safety for emergency cases of shock due to hemorrhage, in which instances the time factor is so urgent that the direct matching must be omitted.

In any event, wherever possible, donor and recipient should belong to the same group.

*The Heredity of the Factors A and B.*—In 1910, von Dungern and Hirschfeld<sup>15</sup> established that the isoagglutinogens A and B are inherited as dominant mendelian properties. In America this significant finding was confirmed by Ottenberg.\* While this explanation of the mechanism of the heredity of the blood groups has been discarded in favor of a more recent theory of triple allelomorphs of Bernstein,<sup>16</sup> the original claim of von Dungern and Hirschfeld, that the property A or B never appears in the children unless they are present in the blood of one or both parents, is in harmony with both theories. As a result of their investigation, they maintained that a basis was furnished to exclude in a certain number of cases individuals as fathers of particular children. In view of the general acceptance of the Bernstein theory, described in 1925, it will not be necessary to describe here the two independent factor theory of von Dungern and Hirschfeld.

Briefly stated, Bernstein believes that the blood group (phenotype) of any individual is determined by the presence at a certain locus, in a particular pair of chromosomes, of any two of the 3 genes A or B or O; the one gene is derived from the father, the other from the mother. Thus, the various possible combination of these 3 genes form the 4 blood groups.

TABLE II

GENOTYPE	PHENOTYPE (BLOOD GROUPS)
OO	O
OA	A
AA	
OB	B
BB	
AB	AB

It is to be noted that, genotypically, the individual is identified by 2 genes, one derived from the paternal and the other from the maternal chromosome.

In the course of the development of the sex cells a reduction division takes place so that the sperms and eggs carry only either the paternal or maternal chromosomes whereas the somatic cells carry both. Thus an individual of blood Group A may be either homozygous (AA) or heterozygous (OA). Serologically

\*See remarks by Epstein, A. A., and Ottenberg, R.: Proc. New York Path. Soc. 8: 117, 1908.

these two types cannot be differentiated, but the children possible from matings with the two sorts of parents differ. Similar relationships hold for Group B individuals.

The various combinations of matings with children possible and children not possible are given in Table III.

TABLE III

	PARENTS	CHILDREN POSSIBLE	CHILDREN NOT POSSIBLE
1.	OxO	O	A, B, AB
2.	OxA	O, A	B, AB
3.	OxB	O, B	A, AB
4.	AxA	O, A	B, AB
5.	BxB	O, B	A, AB
6.	AxB	O, A, B, AB	-----
7.	OxAB	A, B	O, AB
8.	AxAB	A, B, AB	O
9.	BxAB	A, B, AB	O
10.	ABxAB	A, B, AB	O

A few points in the table require elucidation. It may be asked why children of Group O are possible from a mating of AxA parents (or BxB parents). Such children are possible only if both parents are genotypically of the type AO (or BO); and under such conditions the classical 3:1 ratio, i.e., 3 of the dominant A type and 1 of the O (recessive) type can be demonstrated. This genetic cross is given below.

Parents	AOxAO
Gene in sperm or egg	A A or O O
Children genotype	1 AA 2 AO, 1 OO
Children phenotype	3 A's and 1 O

Regardless of the blood of the parent, an individual homozygous to A or B cannot have children in Group O (Genotype OO), since the homozygous parent cannot furnish an O gene to the child. However, this plays no practical rôle since, as already mentioned, the types AO and AA cannot be differentiated from each other.

The other matings which require some analysis are those involving an AB parent. In these matings an AB parent, be it father or mother, cannot have an O child and vice versa. As a corollary, in the mating OxAB, children of the parental types are excluded and only children heterozygous to A and B are possible. These exclusions become obvious from a consideration of the reduction division and the recombinations of the genes to form individuals of the following generation.

Parents	OOxAB
Gene in sperm or egg	O A or B
Children genotype	1 OA, 1 OB
Children phenotype	A, B

This theory has been confirmed in studies on thousands of families by independent investigators working in numerous countries. In any mating, chil-

dren not expected from the theory are illegitimate (indicated by asterisk in Table IV).

TABLE IV  
HEREDITY OF BLOOD GROUPS  
(COMPILED BY LATTES<sup>17</sup>)

PARENTS	NO. OF MATINGS	NO. OF CHILDREN IN GROUP			
		O	A	B	AB
1. O x O	1192	2630	15*	2*	0
2. O x A	2535	2256	3021	18*	9*
3. O x B	997	958	11*	1230	1*
4. A x A	1256	476	2364	1*	1*
5. B x B	293	126	0	532	1*
6. A x B	1104	401	791	641	580
7. O x AB	465	38*	571	525	34*
8. A x AB	481	21*	525	253	307
9. B x AB	327	13*	121	306	159
10. AB x AB	67	0	39	42	70
Total	8717	6919	7458	3550	1162

Additional support for the validity of the theory is derived from a statistical consideration of the incidence of the four blood groups and the distribution of the genes in various races. Studies during the Great War by L. and H. Hirschfeld<sup>18</sup> on numerous races, concentrated at the Macedonian front, have revealed the highly interesting fact that the incidence of the blood groups varies in different races. From a consideration of the frequency of the 3 genes A, B, and O (respectively as p, q, and r), it follows that, given the frequency of any two of the blood types, A or B or O, in any population, a theoretical value of the remaining two types can be obtained to which the observed values correspond very well.

From these considerations, the following statements may be submitted as significant from the medicolegal point of view:

1. Those children having the property A or B not present in the blood of either parent are illegitimate.

2. Children in Group O are not possible in any mating involving an AB parent; children in Group AB are not possible in a mating involving an O parent.

In view of two apparently authentic cases recently described which are exceptional to Rule 2, i.e., Mother AB, child Group O, Landsteiner, Schiff, Wiener and the writer hold the very conservative opinion that, in such cases, there is a very great probability of illegitimacy in contrast to exceptions to Rule 1 in which an unqualified decision of illegitimacy may be rendered.\*

The frequency with which an exclusion of paternity can be made in the U. S. with the factors A and B has been estimated by Wiener to be about 18 per cent. This figure does not differ much from that for European countries. Table V actually shows the frequency with which an exclusion has been made in practice in various countries, in which the courts of justice accept these tests as evidence. Of course, the difference in the theoretical value and the value actually obtained is found in the fact that not every accused man is innocent.

\*For an explanation of these exceptional cases, compatible with the Bernstein theory, see Levine.<sup>19</sup>

TABLE V  
(MODIFIED AFTER SCHIFF<sup>21</sup>)

	NUMBER OF CASES	PATERNITY EXCLUSIONS	PERCENTAGE OF EXCLUSION
Germany (Schiff)	4519	353	7.8
Austria (Werkgartner)	700	63	9.0
Danzig (Pirschel)	600	39	6.5
Denmark (Thomsen)	50	6	12.0
Denmark (Sand)	500	64	12.8
Sweden (Wolff)	259	17	6.6
Norway	37	4	10.8
Switzerland			
Lithuania			
(Cited by Schiff)			
Total	6665	546	8.2%

These tests are significant only for exclusion of paternity and not for detection of the father except in those cases in which it is known definitely that only two men cohabited with the woman. If, in these instances, one of the men can be excluded, then the other individual must be the father (see case of Wiener<sup>22</sup> cited on p. 797).

The blood groups are of great value in detecting cases of interchange of babies at hospitals and recently the tests have been successfully applied in the much discussed Watkins-Bamburger babies in Chicago. With the use of A and B, interchange of infants can be detected in about 50 per cent of all cases (Wiener<sup>23</sup>).

Unfortunately the courts in the United States hesitate to recognize the value derived from an examination of the blood in cases of disputed paternity. However, active measures are being taken in several states to encourage the introduction of evidence based on these tests. Recently, in New York State, Justice Meier Steinbrink rendered a decision, which called for blood tests to settle the question of paternity of a child. Certainly the organization of a forensic section in the American Medical Association will do much to stimulate interest on the part of both physicians and lawyers in this field\*. The subject is already recognized by various American legal authorities; thus Herzog<sup>24</sup> in his textbook, *Medical Jurisprudence*, in 1931 and Wigmore<sup>25</sup> in the 1934 supplement to the treatise on Evidence, discuss the subject at length. Wigmore, the learned authority on Evidence at the Northwestern University School of Law, writes, "At the hands of expert specialists—but of specialists only—the tests ought to be more widely known and used in this country, within the limited scope of their probative value." In view of the facts cited above and the successful experiences of the European courts of justice, it is safe to assume that the American courts will soon follow the precedent already established.

Past experience has shown that the popular knowledge of the existence of these tests has a great psychologic value. The Wurtemberg Ministry of Justice prevents the mother from offering testimony which will not be in harmony with the results of the blood group examinations, by ordering the blood tests before the mother is placed under oath. In certain cases men have agreed to accept children as their own and to pay for their support, when tests revealed that the in-

\*Landsteiner, K.: J. A. M. A. 103: 1041, 1934.

dividual suspected by them was not the father. Wiener cites a case in which a husband sought a divorce on the ground that he was not the father of his wife's child. The husband urged blood tests and his wife gladly came for the examination, but the husband at the last minute refused to submit to the tests he originally requested.

*Blood Stains in Criminal Cases.*—In 1903, Landsteiner and Richter<sup>26</sup> demonstrated the stability of the agglutinogens and the agglutinins present in blood stains to various environmental conditions, such as drying and aging. Thus the group of a blood stain can be reconstructed by determining (1) which isoagglutinins are still present in watery extracts of the blood stain and (2) which isoagglutinogens are detectable as tested by the capacity of the stroma to absorb certain quantities of the isoagglutinins. Only minute quantities of the blood stains are required for these tests. Of the two properties, the isoagglutinogens are the more stable, although the writer was able to detect minute quantities of both anti-A and anti-B in a blood stain of Group O that was four years old.

The stain may be found on clothing, metal implement or wood. To test for isoagglutinins, small quantities of the scraped-off material are extracted in a few drops of distilled water; the extraction is allowed to continue for about one or two hours in the cold (to prevent bacterial contamination). After preliminary tests with a human precipitating immune serum have established the human origin of the stain, the centrifuged and hemoglobin-colored supernatant fluid is then tested with suspensions of known cells of Groups A, B, and also O. The latter blood is included as a necessary precaution because elements other than blood may be present in the extracts which may conceivably have a non-specific agglutinating action on any blood. The results, therefore, are significant if there is no action on blood O, but action on bloods A or B or both. Absorption experiments are then carried out with the extracted sediment to determine the presence or absence of isoagglutinable properties A or B. If, after contact with the stain, the activity of isoagglutinin anti-A is considerably diminished as tested with blood A, then this is proof for the presence of the property A in the stain. The evidence is strengthened by demonstrating isoagglutinin anti-B in the extracts, but the demonstration of the isoagglutininogen is usually more successful on account of its greater stability.

The value of the information obtained from these investigations is seen from the following contrasting cases cited from the literature. Martin and Roehaix<sup>27</sup> reported a murder case in which the property A was shown to be present in the dried blood stain. The suspected individual claimed that the blood stain resulted from a self-inflicted injury. Since he belonged to Group O, his testimony was shown to be false. Popoff<sup>28</sup> described a case in which two men were falsely accused of murder of a woman found with stab wounds in the face. A sharp blood-stained implement was found in their possession which apparently fitted the wounds that penetrated the face. The two men claimed that the blood on the implements resulted from a fight with other individuals, that did not involve the woman. The claims of the accused men were substantiated since the stains showed the property B, while the woman belonged to Group O; the real murderers, furthermore, were later apprehended.

Since the blood factors, as stated above, are present in saliva, cigarette stubs, or envelopes found at the scene of a crime may be investigated; similarly in cases of rape, clothing stained with semen may be available for studies on the blood factors.

The chief protagonists of this aspect are Lattes<sup>29</sup> of Modena, Italy, and Popoff in Russia. These authors recently collected a series of murder cases in some of which the testimony offered by witnesses as to the origin of a blood stain was shown to be incompatible with the result of the tests. On the strength of this evidence the witness, in several instances, confessed that his testimony was false and that he participated in the crime.

*The Properties M and N.*—Before discussing these properties, which are demonstrable by means of immune agglutinins, it may be of advantage to illustrate with the blood groups A and B, the general procedure of the technique.

The factors A and B may be demonstrated also by means of several other reagents, namely normal animal serums (which contain heteroagglutinins not only for blood of Groups A and B, but also for human blood in general), and immune serums derived from rabbits after injection of bloods A and B. The latter of the two methods is preferable since potent reagents are obtained. They are, however, less convenient to work with than the normal isoantibodies, because very frequently anti-A or anti-B immune serums contain other antibodies which react on all human bloods (species antibodies). A preliminary absorption of the diluted serums with bloods of Group O is required to remove the species agglutinins. The supernatant fluid may then be employed to diagnose bloods A or B.

With the aid of this general technique, Landsteiner and myself<sup>30</sup> described in 1928 several new agglutinable blood properties, the most significant of which are termed M and N. These factors are demonstrable by means of specific immune agglutinating serums derived, as a rule, from rabbits in a manner similar to that described above for A and B. With one exception normal antibodies specific for M and N are not present in normal human serum. On examining numerous bloods of any of the four blood groups, with the two sorts of serums, previously absorbed with a suitable blood lacking the factor in question, 3 types are found, namely,  $M^+ N^+$ ,  $M^+ N^-$  and  $M^- N^+$ . The incidences of the three types in the white population are 50, 25, 25 per cent. Although thousands of bloods have been examined by several workers, a blood of the type  $M^- N^-$  has never been observed. Accordingly, at least 12 sorts of human blood are readily demonstrated by means of reagents for A, B, M and N. The antisera for M can be produced by injecting rabbits with bloods preferably of Group O and of the type  $M^+ N^-$ ; those for N, by injecting Group O blood of the type  $M^- N^+$ . Injections are continued until preliminary absorption tests with suitable bloods show that antisera with potent specific antibodies are produced. The rabbits are bled, and serums collected, filtered, and under such conditions they remain potent for several years.

Since these factors cannot, as a rule, be demonstrated by means of normal human serums, they play no rôle in the successful outcome of transfusions. However, some interesting observations have been made on the length of life of



the transfused corpuscle by studying selected cases of donor and recipient of the same group, but differing in M. In one instance of recipient M<sup>-</sup> and donor M<sup>+</sup>, the M<sup>+</sup> blood could still be found circulating in the blood of the recipient after an interval of one hundred days following the transfusion.<sup>31</sup>

Studies by numerous investigators on the heredity of M and N confirmed the theory of Landsteiner and Levine<sup>32</sup> as to the mechanism of the heredity. According to these authors, there are two genes M and N, the distribution of which in various combinations gives the three types serologically demonstrable (phenotypes).

PHENOTYPE		GENOTYPE
M <sup>+</sup> N <sup>-</sup>	(M)	MM (homozygous)
M <sup>-</sup> N <sup>+</sup>	(N)	NN (homozygous)
M <sup>+</sup> N <sup>+</sup>	(MN)	MN (heterozygous)

The theory demands the following incidence of children in the six possible matings (Table VI).

TABLE VI

HEREDITY OF THE FACTORS M AND N ACCORDING TO THE THEORY OF LANDSTEINER AND LEVINE

MATING NO.	TYPES OF PARENTS	PER CENT OF CHILDREN OF TYPES		
		M	N	MN
1	MxM	100	0	0
2	NxN	0	100	0
3	MxN	0	0	100
4	MxMN	50	0	50
5	NxMN	0	50	50
6	MNxMN	25	25	50

In an examination of 671 families with 2,365 children the following results were obtained as given in Table VII, which includes the studies of Landsteiner and Levine, Wiener and Vaisberg, Schiff, Schiff and Sasaki, Thomsen and Clausen, and Lattes and Blaurock.

TABLE VII<sup>1</sup>

TYPE OF PARENTS	NUMBER OF CHILDREN OF TYPES			
	M	N	MN	TOTAL
MxM	203	0	1*	204
NxN	0	107	0	107
MxN	0	2*	205	207
MxMN	317	3*	362	682
NxMN	2*	230	268	550
MNxMN	149	135	331	615
Totals	671	527	1167	2365

<sup>1</sup>Data collected from the literature by Wiener.

On comparing the combined figures obtained by all these investigators, working in four different countries, with that expected from the theory, a very good fit is found. The eight children not expected from the theory are considered illegitimate (indicated by asterisk in Table VII).

For convenience Table VI is rearranged to point out in each mating the children possible and those to be considered illegitimate.

TABLE VIII

MATING	TYPE OF PARENTS	CHILDREN POSSIBLE	CHILDREN ILLEGITIMATE
1	MxM	M	MN, N
2	NxN	N	MN, M
3	MxN	MN	M, N
4	MxMN	M, MN	N
5	NxMN	N, MN	M
6	MNxMN	N, N, MN	--

From the theory given below, the following rules may be laid down:

a. The factor M or N cannot appear in the blood of a child unless it is present in the blood of one or both parents.

b. In matings where both parents are homozygous to M ( $M^+ N^-$ ), all children are of the same type; the other two types are excluded. The same rule holds for matings  $NxN$  (matings 1 and 2).

c. In case of one parent homozygous to M and the other homozygous to N, 100 per cent of the children are of the type MN ( $M^+ N^+$ ), the parental types are excluded (mating 3).

d. In matings where one parent is homozygous and the other heterozygous, children of the parental types are to be expected in a ratio of 50:50 (matings 4 and 5).

e. In matings with both parents heterozygous (MN), children of the three types are possible (mating 6). This is the only mating in which no exclusion is obtained.

The genetic analysis of mating number 3 is given as an example; the other matings may be analyzed in a similar manner.

Parent	MMxNN
Geno in sperm and egg	M N
Children	
Genotype	MN
Phenotype	MN

As a corollary to the statements made above, the following may be stated: namely, a parent of the Type M (matings 1, 3, and 4) cannot have a child of the Type N; similarly, a parent of Type N (matings 2, 3, and 5) cannot have a child of Type M. The reason for this exclusion lies in the fact that the parent, being homozygous to one gene (M), cannot contribute the other gene required (N). This fact makes it possible to render decisions of exclusion of paternity in a good proportion of cases; namely, the first 5 matings, from an examination of the blood of child and only one of the parents. In this respect the MN system is far superior to the A and B system. Thus in 7 of the 8 cases of illegitimacy (matings 3, 4, and 5) the father's blood and not the mother's blood is of a type incompatible with that of the child. In these 7 cases the child's blood is of the homozygous type; in the eighth case the child's blood is heterozygous.

Wiener has calculated that a decision of exclusion of paternity with the use of factors M and N can be made in 18 per cent of all cases. Since the factors M and N are independent of A and B, the application of the 4 factors results in an exclusion in about one-third of all cases.

Employing all 4 blood factors, Schiff, who has had the greatest experience in this branch of forensic medicine, obtained an exclusion of 134 times (14.7 per cent) in an examination of 911 cases. These values are 40 to 50 per cent of the maximum since, as already mentioned, not every accused man is innocent.

It must be added, however, that the technique for demonstrating M and N is far more involved than that for A and B and that it presupposes an intimate knowledge of immunologic principles. When the American courts finally agree to recognize the value of these procedures, the policy should be employed, of relegating the tests to recognized serologists, preferably those connected either with universities or the larger hospitals.

A few cases where M and N have been employed in America to exclude paternity have been reported by Wiener.<sup>22</sup> As an example the following instance may be cited, in which exclusions were made with both the AB and the MN systems. The bloods of the three adults and the three children whose legitimacy was questioned were the following:

BLOOD OF	GROUP	TYPE
Husband	O	MN
Accused man	A	N
Wife	O	MN
First Child	O	MN
Second Child	O	M
Third Child	A	N

Apparently the third child, in group A, cannot be the husband's because O x O cannot beget an A, but can be the child of the accused; the second child cannot be the lover's since the latter with the property N cannot have a child M.

A case recently examined by me is instructive from several points of view. Only the bloods of the child and alleged father were available for examination. Tests revealed the following: alleged father B,  $M^+ N^-$ ; child A,  $M^+ N^+$ . In this instance, no exclusion can be made without an examination of the mother's blood, which can be only in groups A or AB and with regard to M and N either  $M^+ N^+$  (MN) or  $M^- N^+$  (N). The mother cannot belong to group O or B since O x B or B x B cannot give an A child; she cannot have the property  $M^+ N^-$  since in mating  $M^+ N^-$  x  $M^+ N^-$ , the child  $M^+ N^+$  is excluded. In view of the fact that Group O is present in about 45 per cent of all white people and the type  $M^+ N^-$  in about 25 or 30 per cent, there is a very great probability of an exclusion. If the child were of the type  $M^- N^+$ , the exclusion could be made without an examination of the mother's blood.

The properties M and N may also be used to detect instances of interchange of babies. The demonstration that M and N remain stable in dried blood, furnishes the basis for the forensic application also of these factors in criminal cases involving blood stains.

Very brief mention may be made of several other factors; namely, those describing the subgroups of Groups A and AB and the factor P.<sup>33, 34</sup> The subgroups may be demonstrated most conveniently with the aid of those rare human serums of Groups AB or A which contain the specific atypical agglutinins anti-A<sup>1</sup> and anti-A<sup>2</sup> (see page 787). These antibodies without any previous absorption select the two sorts of bloods; the antibody for A<sup>2</sup> acting also on all bloods of Group O. Also these properties are inherited, but further study is required before these factors can be employed with safety in cases of exclusion of paternity.

The factor P may be demonstrated by means of more or less related antibodies present rarely in some human serums containing the atypical specific agglutinin, and more frequently in normal animal serums. The property may be demonstrated also by means of specific immune antibodies derived from the rabbit. This property, like A<sup>1</sup> and A<sup>2</sup>, is not as well defined as A or M, but studies by Landsteiner and Levine show also that the factor P is distinctly inherited.

Our knowledge of individual differences of human blood has been considerably extended in more recent years. Encouraged by the successful experiences of several European workers in the application of this knowledge, a number of scientists and physicians in America are making organized efforts to educate the American courts to accept the scientific principles discussed, as testimony in both bastardy proceedings and in cases involving blood stains. An essential preliminary measure required is the passage of a law to compel individuals to submit to the blood tests.

It is difficult to estimate the number of individuals who unwittingly are paying support for children not their own, but whenever possible, justice should be meted out. And finally, is it not preferable to speak of illegitimate parents rather than illegitimate children?

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#### ADDENDUM

Recently New York State passed laws empowering the courts to order blood tests in cases of disputed paternity. Undoubtedly many states will in the next few years take active measures to pass laws of a similar nature.

As a readily available source for workers interested in promoting the use of these tests, the laws as passed by the New York State Legislature are here appended.

A bill similar to that given below, and sponsored by myself, was recently submitted to the Wisconsin legislative bodies. It was framed with the aid of Prof. Bunn of the University of Wisconsin Law School and has the support of Dean Lloyd K. Garrison. Such legislation is encouraged by the American Medical Association (*J. A. M. A.* 103: 46, 1934).

#### STATE OF NEW YORK

No. 124      Int. 123

#### IN ASSEMBLY

January 9, 1935.

Introduced by MR. BREITBART—read once and referred to the Committee on Codes.

#### AN ACT

To amend the civil practice act, in relation to empowering the court to order the making of blood tests.

*The People of the State of New York, represented in Senate and Assembly, do enact as follows:*

Section 1. The civil practice act is hereby amended by inserting therein a new section, to be section three hundred six-a, to read as follows:

306-a. Blood tests. Wherever it shall be relevant to the prosecution or defense of an action, the court, by order, shall direct any party to the action and the child of any such party to submit to one or more blood tests, to be made by duly qualified physicians and under such restrictions and directions, as to the court or judge shall seem proper. The order for such blood tests may also direct that the testimony of the persons so examined may be taken by deposition pursuant to this article.

2. This act shall take effect immediately.

## STATE OF NEW YORK

Nos. 167, 682      Int. 166

## IN ASSEMBLY

January 9, 1935.

Introduced by MR. BREITBART—read once and referred to the Committee on Codes—committee discharged, bill amended, ordered reprinted as amended and recommitted to said committee.

## AN ACT

To amend the inferior criminal courts act of the city of New York, in relation to empowering the court to direct the making of blood-grouping tests and permitting the results thereof to be received in evidence.

*To People of the State of New York, represented in Senate and Assembly, do enact as follows:*

Section 1. Section sixty-seven of article five of chapter six hundred fifty-nine of the laws of nineteen hundred ten, entitled "An act in relation to the inferior courts of criminal jurisdiction in the city of New York, defining their powers and jurisdiction and providing for their officers," as such section and article were thus restated and renumbered by chapter seven hundred forty-six of the laws of nineteen hundred thirty-three, is hereby amended by inserting therein a new subdivision, to be subdivision one-a, to read as follows:

## 2

1-a. The court, on motion of the defendant, shall order the making of one or more blood-grouping tests by a duly qualified physician and the results thereof may be received in evidence.

2. This act shall take effect immediately.

# THE INTESTINAL TOXEMIA SYNDROME\*

## TREATMENT WITH KAOLIN

C. A. MILLS, M.D., CINCINNATI, OHIO

CERTAIN new phases of the intestinal toxemia syndrome have presented themselves in recent years and seem of sufficient importance to warrant writing this article, even though this paper is on a subject that has been so badly overworked in medical literature of previous decades. The fact that certain commercial concerns have widely exploited this field gives the medical profession no warrant to neglect it. In some regions intestinal putrefaction, with the body disturbances resulting from the foul absorption, forms a real health problem. This is particularly true in the two major storm areas of the earth, a large part of North America and West Central Europe including the British Isles. In the tropics, subtropics, and Orient putrefaction in the intestine occurs much less frequently. In fact it is not unusual in China to see natives who have a bowel movement only every two or three weeks but are still entirely free from any signs of toxic absorption.

Why putrefaction should be worse in the intestines of people living in stormy regions is not yet known. There is a strong suspicion that sudden falls in barometric pressure cause body tissues to take up water and the secretion of digestive juices to lessen. This point is under investigation at present. Clinical observation, however, has given the impression that putrefaction does tend to flare up in the bowel on those days when the barometric pressure is low or falling. Whether this is due to changes in secretion of the digestive juices remains for the future to answer. Regardless of the causes of the putrefaction, we of northern United States have it as one of the important sources of discomfort, loss of efficiency, and eventual disease along certain lines. For the last six years I have given particular attention to this problem and have found that elimination of putrefaction in many cases produces surprising consequences. The agent found most certain in action and economical in cost is kaolin, administration in a syrupy suspension. The following prescription is used. If cascara or antispasmodics are needed they may replace a corresponding volume of water.

R Kaolin	̄ VI
Water	̄ IV
Syrup simplex q.s.	̄ XII

Dosage: ½ ounce twice a day before meals.

As a result of clinical observation the distinct impression has arisen that absorption of putrefactive products from a foul colon, and their excretion

\*From the Department of Experimental Medicine, University of Cincinnati.  
Received for publication, October 1, 1931.

through the saliva and sweat glands, is directly responsible for the lowering of resistance to infection in the mouth and skin. Confirmation of this impression rests upon the marked manner in which pustular acne and early pyorrhea respond to kaolin administration. Acne of the skin has long been known to be associated with intestinal toxemia, but its treatment by dietary means has often been unsatisfactory. With kaolin, prompt subsidence almost always occurs, the skin softening and gradually clearing. This is of particular value in children during adolescence, for at this age putrefaction is especially liable to appear and, unless properly treated, is likely to become more or less permanent. Boys are much more afflicted in this way than are girls, and need to be watched more closely. Cases of severe pustular acne over almost the whole body have responded well to kaolin, the lesions beginning to subside within a week and being practically gone in a month without other treatment of any sort.

As regards the mouth, most physicians and investigators have seemed inclined to regard halitosis and pyorrhea as of local origin, due to faulty mouth hygiene. After observing the prompt disappearance of the foul breath and gradual improvement of early pyorrhea as intestinal putrefaction is eliminated, I have been forced to the conclusion that these oral conditions rely primarily on the presence of putrefaction in the bowel. The saliva in these cases contains indican, giving a positive test just as does the urine, although to a lesser degree. Lowering of resistance to infection may result from the gums being constantly bathed in this concentrated fecal extract. The fecal odor to the breath in such cases is a natural consequence of rapid evaporation of the saliva, especially during speech. At night, when water intake is stopped, greatest concentration takes place, with the resulting "dark brown taste." Whether certain types of dental caries rest on this same basis is still uncertain. It is, however, a point well worthy of close investigation.

The intestinal toxemia syndrome, then, in addition to the usual foul gas production, indigestion, headaches, and general biliousness, includes these other conditions; pustular acne of the skin, halitosis and much pyorrhea, and perhaps some types of dental caries. There are also the nervous and vascular disturbances caused by the absorbed products, nervousness, insomnia, and disturbed sleep, and in a great many cases marked vascular spasm. Where a tendency to hypertension exists, putrefaction adds greatly to the spastic condition of the vessels, so that considerable reductions in blood pressure often occur after kaolin administration.

Nothing new is being proposed in this use of kaolin. Its effectiveness as a selective adsorptive reagent for the alkaline amines and putrefactive bacteria was demonstrated years ago. The medical profession is not benefiting by this knowledge, however, for few pharmacists carry kaolin in stock except in Cincinnati where its use has been actively advocated for the past six years. The prompt effectiveness and low cost strongly recommended its widespread use by the medical profession in our northern states where intestinal putrefaction is so prevalent. School and college health authorities should make free use of it. Many unnecessarily expensive preparations are on the market. The Cincinnati Hospital buys a good grade of kaolin for 4 cents a pound in barrel lots, and one



pound lasts a patient a month. Elimination of putrefaction need not, therefore, be an expensive treatment, especially if the mixture be prepared at home. The treatment cost is an important item, since people needing the kaolin usually continue to need it off and on at all seasons. Permanent cessation of putrefaction is usually not obtained.

For some patients kaolin is somewhat constipating, and, for a few individuals with marked spasticity of the colon, distinctly irritating. By its reduction in bacterial growth, it tends to lessen the stool bulk and the need for frequent evacuation. No cases of fecal impaction or fecalith formation resulting from it have been seen in six years' use, so I feel that the caution promulgated a few years ago<sup>2</sup> can be disregarded. Numerous references are available to substantiate the claims for beneficial kaolin effects in diarrheal or putrefactive disturbances of the bowel. Only one case of fecalith formation and fatal impaction has been reported,<sup>3</sup> and in this case kaolin was given in daily single doses of 100 gm., presumably suspended in water. Even then its immediate action in stopping a severe bloody diarrhea was promptly effective, the signs of intestinal obstruction developing a week after kaolin administration was discontinued. For the Council on Pharmacy and Chemistry of the American Medical Association to rule against kaolin preparations and their therapeutic use on the basis of this one fatality seems hardly justified. On such a basis action should long ago have been taken against insulin for the many deaths it has caused. Administered in a syrupy suspension, kaolin shows little tendency to clumping in the bowel and is more effective in depressing putrefaction than when given suspended in water.

It must be stressed here that frequency of bowel movements and intestinal toxemia need not be at all associated. Individuals may go for weeks without an evacuation and still show no signs of toxic absorption, provided putrefactive activity is at low ebb. On the other hand we see many patients with a putrefactive diarrhea in whom no relief from the toxicity is obtained by eight to ten bowel movements a day. Naturally, if putrefaction is prevalent, its effects will be accentuated by constipation. Many people, however, go to unnecessary trouble to secure a daily evacuation simply because it is decreed by custom. This custom originated in the cool stormy areas where putrefaction is worse and probably represents a necessary development for the mass of humanity in these regions.

It would seem that the medical profession should give more attention to this question of foul colonic contents and adopt some simple, inexpensive method of treatment that the patient can use the year round. The mere presence of aene or halitosis is usually direct evidence that kaolin, or a similarly acting substance, is needed. Dietary treatment is much less certain in its results, is slower, and is less likely to be adhered to by many patients. If, as suggested here from close clinical observation, pyorrhea and much of dental caries can be eliminated or prevented by proper colonic hygiene, then a great forward step will have been taken in the care of the mouth. It has been truly striking to witness the marked diminution in tartar deposit on the teeth and the general feeling of mouth cleanliness that results from elimination of putrefaction in the bowel.

A few illustrative case histories are briefly summarized below. Most physicians are aware of the symptoms attending intestinal putrefaction. The impressions set forth in these pages have resulted from close observation of many hundreds of patients during the past few years. What is particularly desired in this article is to stress the close connection between oral and colonic hygiene, and the probable rôle played by the excretion of the absorbed putrefactive products through the saliva and sweat glands. It scarcely seems necessary to emphasize the relief from gastroenteric disturbances that results from cessation of putrefaction. Medical literature of twenty years ago lay particular emphasis on that aspect of the problem.

#### CASE REPORTS

CASE 1.—A white man, forty-two years old, came to the Medical Clinic Feb. 1, 1934, for treatment of a severe pustular eruption which had afflicted him for eight years. A few eruptions were scattered over most of the body, but from the waist down they increased greatly in severity and numbers, with large areas of induration, heat, and redness, and one furuncle just above the left knee. Itching was severe and was the reason for the patient's call at the clinic. He had been chronically constipated, with much flatulence, for about ten years. Teeth had all been extracted eight years previously because of severe pyorrhea. One sample of urine showed 1+ sugar, but none was found on subsequent repeated examinations and the fasting blood sugar level was 74 mg. per cent. Kahn test was negative.

Having failed to respond to local skin treatments in the dermatologic clinic, he was placed on kaolin. Marked improvement was evident after one week, the heat and redness largely gone and the itching less troublesome. The lesions continued to fade steadily, with no new ones appearing for the six weeks of observation. At this time the urine test for indican, which had at first been intensively positive, gave only a slight color. The saliva before kaolin therapy had also given a mild positive indican test, but after six weeks was entirely negative.

CASE 2.—Another white man of forty-two years came in complaining of constipation with much foul gas production, headache most of the time, and inability to sleep without disturbing dreams. Hands and feet were cold and cyanotic. General development was good, blood pressure normal. Early pyorrhea and halitosis present. Physical findings otherwise negative. Kaolin administration gave prompt relief from all this patient's symptoms within two weeks.

CASE 3.—A white boy of fifteen years, well developed and otherwise in good health, began at thirteen years to have acne of the face, foul gas production, and an alarming type of rather widespread dental decay. The gums were red and spongy, bleeding easily, while the teeth coated rapidly and the breath was at times foul. Kaolin used at irregular periods has been able to keep down the acne and gas production, while the dental decay has gradually lessened. Gingival tissues regained normal tone and appearance, coating of the teeth ceased, and now at fifteen years the boy rarely needs kaolin. Several times in the past two years, when at camp or away from home, he has returned with all putrefaction symptoms present, but each time his response to kaolin has been prompt.

*In summarizing, then, the following points are to be noted:*

1. A close association seems to exist between oral and colonic hygiene. Putrefactive activity in the colon seems to result in halitosis, excessive tartar deposit and lowered vitality of the gingival tissues. The suggestion is made that this effect may even extend to the teeth and be the basis of much caries.

2. Intestinal putrefaction is also at the basis of much acne of the skin, and the elimination of such putrefaction often brings most rapid and complete disappearance of even the most severe pustular acne.

3. It is suggested that these oral and skin troubles result from the excretion through the saliva and sweat glands of the putrefactive products absorbed into the blood from the colon.

4. The most rapid and certain treatment for intestinal putrefaction is kaolin, administered in a syrupy suspension. This preparation has the added value of low cost, which is important in this type of disturbance that usually requires continued treatment for years.

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# JUVENILE PARETIC NEUROSYPHILIS STUDIES\*

## IX. LABORATORY FINDINGS

WILLIAM C. MENNINGER, M.D., TOPEKA, KAN.

THE laboratory findings in congenital neurosyphilis have been the subject of several studies, and yet there has been no large series of one type of congenital neurosyphilis reported. The following study includes the blood and spinal fluid Wassermann tests, the spinal fluid cytology and the colloidal gold reaction in juvenile paresis. It is one of a series of papers<sup>15</sup> based upon 43 personally studied cases and a survey of 610 cases reported in the literature.

In this total group of 653 cases surveyed data are given in 325 cases regarding the serologic findings. In an additional 139 cases postmortem findings corroborated the diagnosis. Table I indicates the laboratory findings in the blood and spinal fluid:

TABLE I  
WASSERMANN AND COLLOIDAL GOLD TESTS IN 325 CASES OF JUVENILE PARESIS

Blood Wassermann positive (In 259 of these the spinal fluid was either positive or not examined; in two, the spinal fluid was negative.)	261 cases
Blood Wassermann negative (In 3 cases the spinal fluid Wassermann was positive, in two it was not examined, and in two it was negative.)	7 cases
Spinal Fluid Wassermann positive (In 269 of these the blood was either positive or not examined; in three it was negative.)	272 cases
Spinal Fluid Wassermann negative (In 2 cases the blood was positive; in two it was not reported, and in two it was negative.)	6 cases
Spinal Fluid Colloidal Gold with marked first zone "typically paretic" curve recorded in	89 cases
Weak first zone or second zone change	15 cases

This table can give us only a rough approximation of the findings, since it is impossible to compare Wassermann tests accurately without giving consideration to the method used in performing it, the amounts of spinal fluid used, etc. Such data were not available in the many individual case reports used in compiling these figures.

\*From the Menninger Clinic.  
Received for publication, September 15, 1934.

*Negative Blood Wassermann Test in Congenital Syphilis.*—The blood Wassermann test is characteristically positive in congenital syphilis. There are, however, a certain percentage of congenital syphilitic individuals who consistently and persistently show a negative Wassermann reaction in the blood. These are of major importance, not numerically, but in establishing the diagnosis in the presence of negative findings. It is known to be negative frequently for the first two months of life (Ross and Wright,<sup>23</sup> Fordyce and Rosen<sup>6</sup>) and it has been repeatedly shown to fade out as the child grows older (Atwood,<sup>1</sup> Dean,<sup>3</sup> Thomsen et al.<sup>26</sup>). But there are the opinions of many writers that the Wassermann is frequently negative: Kolmer<sup>9</sup> states that "alone is not reliable and is prone to be negative"; Moleen<sup>18</sup> finds it "negative in many very definite cases"; Stoll<sup>25</sup> records it as "often negative"; Leredde<sup>10</sup> says that the "serum is habitually negative"; DuBois<sup>4</sup> believes it is "almost always negative"; and Fischl<sup>5</sup> states it is "unreliable." There is a more conservative group who record the serum Wassermann as usually positive but occasionally definitely negative: Cooke and Jeans,<sup>2</sup> Lespinasse,<sup>11</sup> and Myerson.<sup>19</sup>

A few statistical studies indicate the variation in the findings of different observers, which in part may be due to a difference in the age groups studied (Table II).

TABLE II  
WASSERMANN TEST IN BLOOD IN CONGENITAL SYPHILIS

YEAR	OBSERVER	REFERENCE	NO. OF CASES	PER CENT NEGATIVE BLOOD WASSERMANN
1916	Veeder	Am. J. M. Sc. 152: 522, 1916	128	4.0
1919	Craig	Wassermann Test, St. Louis, 1919, The C. V. Mosby Co., p. 114	128	17.8
1919	Jeans	Am. J. Dis. Child. 18: 173, 1919	214	0.5
1924	Bournhour	Thesis, Toulouse, 1924	—	63.0
1924	O'Leary	Minn. Med. 7: 651, 1924	—	30.0
1925	von Gutfeld	Arch. f. Kinderh. 75: 302; 76: 13, 1925	155	0.0
1933	Smith	Bull. Johns Hopkins Hosp. 53: 231, 1933	462	8.0

From these studies, as well as a previous study by Karl A. Menninger and myself,<sup>14</sup> I conclude that congenital syphilis may be present in a small percentage of cases with negative serum Wassermann reactions.

*Negative Blood Wassermann in Paretic Neurosyphilis.*—A negative blood Wassermann test occurs in between 5 and 10 per cent of all untreated cases of (adult) general paresis. In a recent study, Bromberg and I<sup>16</sup> reported on the negative blood findings in 500 cases of neurosyphilis with positive spinal fluid Wassermann tests. Approximately 30 per cent of that group had negative blood Wassermann tests, and of 38 cases of general paresis included, eight had negative blood Wassermann tests (only one of these had been untreated). In that study, we reviewed the findings of some 30-odd investigators in this field, and found a small, but constant, percentage of cases with negative blood serology.

*Negative Blood Wassermann in Juvenile Paresis.*—In the present study seven cases are reported with negative blood serology; the spinal fluid find-

ings in these cases were as follows: Leonard,<sup>27</sup> 1915, in attempting to test the spinal fluid only obtained dry taps; Southard and Solomon's<sup>27</sup> case (p. 298) showed a positive spinal fluid; Klauder and Solomon's,<sup>8</sup> 1923, case showed positive spinal fluid; Potter<sup>22</sup> 1929 reported two cases, one with positive and one with negative spinal fluid Wassermann tests; Schmidt-Kraepelin's<sup>24</sup> case showed an essentially negative spinal fluid; and in one of my own the spinal fluid was not examined.

From the fact that a negative blood Wassermann is the occasional finding both in congenital syphilis and in general paresis, there is no question that the blood may be negative in juvenile paresis. It may be due in part to previous treatment, in part to a low virulence of the infecting organism, and in part to a fading of the antibodies with increase in age of the patient.

*Negative Spinal Fluid Wassermann in Congenital Syphilis.*—It would hardly be expected that the spinal fluid of congenital syphilis cases would be positive, although it is positive in a surprisingly large percentage of cases even without any signs of clinical neurosyphilis. This is shown in Table III, which gives the opinions and findings of a number of observers.

TABLE III  
FREQUENCY OF ABNORMAL FINDING IN THE SPINAL FLUID IN CONGENITAL SYPHILIS

YEAR	OBSERVER	REFERENCE	NO. OF CASES	PERCENTAGE ABNORMAL SPINAL FLUID
1917	With	Brain 40: 403, 1917	26	31.0
1919	Jeans	Am. J. Dis. Child. 18: 173, 1919	214	66.0
1921	Brewer	Deutsche med. Wchnschr. 27: 1297, 1921	—	73.2
1921	Kingery	J. A. M. A. 76: 12, 1921	52	28.8
1923	Tezner	Monatschr. f. Kinderh. 26: 49, 1923	83	43.3
1923	Frank	Monatschr. f. Kinderh. 25: 136, 1923	18	33.0
1924	Arena	Pediatrics 32: 465, 1924	31	22.6
1924	Bournhoure	Thesis, Toulouse	—	52.0
1924	Solomon	Human Cerebrospinal Fluid, New York, 1926, Hoebner, p. 415	—	20-30.0
1925	von Gutfeld	Arch. f. Kinderh. 75: 302; 76: 13, 1925	155	10.0
1926	Ahman	Acta Paediat. 6: 1, 1926	71	17.0
1931	Cregor	Arch. Derm. & Syph. 24: 732, 1931	103	28.0

One is impressed from these figures with the high frequency of spinal fluid changes in congenital syphilis, and yet the majority of cases do not show an involvement of the central nervous system clinically. That a positive spinal fluid Wassermann may be associated with a negative blood Wassermann finding is shown in the figures of Cregor (cited above) who found 2.5 per cent of his 103 cases with this situation.

*Negative Spinal Fluid Wassermann in General Paresis.*—In a previous study of this subject, K. A. Menninger and I<sup>13</sup> reported the findings in 154 cases of acquired general paresis, in which 94.8 per cent showed a positive spinal fluid Wassermann test. In that paper we reviewed the findings of many other workers establishing a diagnosis of general paresis in the absence of positive spinal fluid findings. Nonne<sup>20</sup> proposed a classification of the types of neurosyphilis with negative serology, which was later expanded by Klauder and

Solomon,<sup>3</sup> and it is generally agreed that clinical neurosyphilis may be present without the spinal fluid Wassermann test being positive.

*Negative Spinal Fluid Wassermann in Juvenile Paresis and Congenital Neurosyphilis.*—In the present survey of juvenile paresis, there are six cases reported with negative spinal fluid Wassermann tests: Huguet<sup>27</sup> 1913, Kleinberger<sup>27</sup> 1919 (the blood is not given), Potter<sup>22</sup> three cases (two with positive serum Wassermann tests and one with a negative serum Wassermann), and Schmidt-Kraepelin,<sup>24</sup> whose case had essentially negative blood and spinal fluid. In two additional cases she reported that the spinal fluid was negative using small quantities, but positive with 1 c.c. of fluid.

That a negative spinal fluid finding may occur in congenital neurosyphilis, is indicated by various observers: Masten<sup>12</sup> found ten cases of clinical neurosyphilis out of 20 examined to have negative spinal fluid Wassermann tests. Higoumenakis<sup>7</sup> studied a group of nine cases, reporting negative spinal fluid Wassermanns even in the presence of hemiplegia or other nervous system lesions. Potter<sup>22</sup> has presented three cases of juvenile paresis with negative spinal fluid serology, as well as expressing the opinion, supporting my own, that juvenile paresis might be definitely present without the positive spinal fluid Wassermann.

A case of juvenile taboparesis that I recently examined at the Public Health Institute, Chicago, illustrates this type of entirely seronegative congenital neurosyphilis.

CASE 29.\*—A male, aged twenty years. The mother had been treated for syphilis for the last ten years. The patient was an only child; he had convulsions during his first year of life, but these did not reappear until fifteen years of age. He was retarded in growth, both physically and mentally. He attended school for four years, continually failed, and was tutored for two years. He began having grand mal attacks at the age of fifteen years, and began antisyphilitic therapy, despite a negative serology. At nineteen he had an operation, supposedly for appendicitis, associated with abdominal pain and induced vomiting. The convulsions which lasted about ten minutes had occurred irregularly, and at times had been replaced by combative, irritable, irrational behavior. Petit mal attacks were more frequent. In addition to these, and without apparent association with them, he had attacks of intense pain in the lower abdomen about once a month, which lasted several hours. Antisyphilitic treatment had been very irregular. *Physically:* He was a tall, extremely emaciated childish lad. *Neurologically* he showed absolute pupil fixation, slight right seventh nerve weakness, a fine tremor of the tongue and extended hands, and moderately increased reflexes. *Stigmata:* He showed grossly misshapen teeth, high arched palate, winged scapulae, marked asymmetrical shaped chest, scoliosis and saber shins. *Mentally* he was childish, made scrapbooks and read children's stories; he had a very meager fund of general knowledge, was unable to do simple calculation, and was exceedingly dependent on his mother. *Laboratory:* Repeated blood Wassermann tests were negative, as

\*Cases 1 to 28 are reported in the previous papers of this series.

was the spinal fluid. *Course:* During a period of two months' observation while on bismuth and tryparsamide, both the convulsive attacks and gastric crises had become noticeably less frequent.

From personal studies, as well as the support from the cases cited in the literature, I am sure that one may find clinical congenital neurosyphilis, and specifically juvenile paresis, with a negative Wassermann test in the spinal fluid. These are rare numerically, but it is of major importance to recognize such a case clinically. Whether it may be due to a low virulence of the infecting organism, a poor formation of antibodies by the individual, a change effected by the age of the person, or by possible unknown amounts and effects of treatment, is entirely speculative. That it occurs is definite.

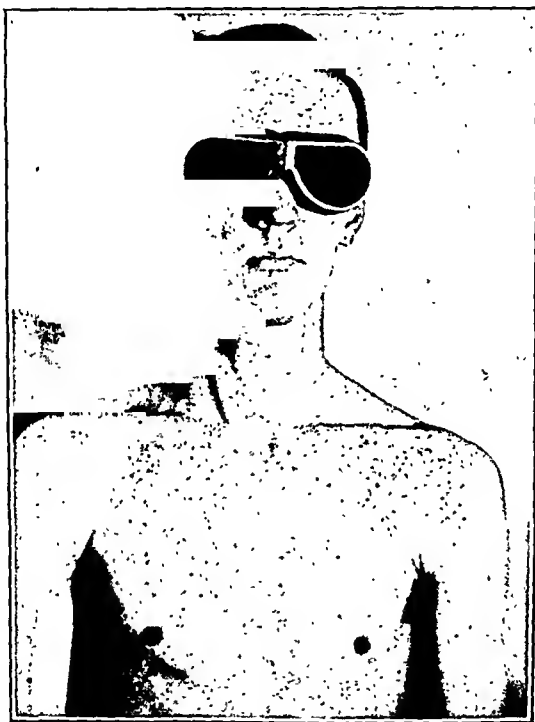


Fig. 1.—Case 29 with negative serum and spinal fluid Wassermann reaction.

*Spinal Fluid Cell Picture.*—The cellular reaction in juvenile paresis is no different from that seen in the acquired type. In the majority of cases, there is an increase in the number of cells, which are characteristically lymphocytes. There may, however, be no increase in cells, and several cases are reported having no cells in the spinal fluid. In the entire group of cases, there are 24 instances, when the cells numbered over 100 per c.mm., and there are six instances with over 200. In one instance they numbered 310 (Strecker and Ebaugh, 1925).

*Colloidal Gold Reaction.*—As indicated in Table I the colloidal gold reaction was a strong first zone, so-called paretic, in 89 cases. In an additional fifteen, the change was either a weak first zone curve, or a change in the second zone ("syphilitic" type of curve). In a recent study in acquired



neurosyphilis in 500 cases, Bromberg and I<sup>17</sup> found that no specific change in the colloidal gold curve could be regarded as diagnostic for a definite clinical type of syphilis, a fact well recognized, and recently stressed by Novy.<sup>21</sup> Consequently, it is hardly to be expected that all cases of juvenile paresis should show a "paretic" type of curve, and the fact that a case does not, in no way contradicts the diagnosis.

#### SUMMARY AND CONCLUSIONS

1. The blood Wassermann reaction was reported negative in seven cases of juvenile paretic neurosyphilis; such a finding occurs in at least 8 per cent of all forms of late congenital syphilis; a negative blood Wassermann occurs in about 5 per cent of general paresis.

2. A negative spinal fluid Wassermann test is reported in six well-established cases of juvenile paresis; such a finding has been repeatedly reported in congenital neurosyphilis, and occurs in from 3 to 5 per cent of all cases of general paresis.

3. In the presence of sufficient evidence from the history, stigmas, and clinical signs and symptoms, a diagnosis of juvenile paretic neurosyphilis is justified, when the blood and spinal fluid Wassermann tests may be negative. The cause for this negative serology is unknown: it may be due to age changes in the individual, to previous treatment, or to low virulence of the infecting organism.

4. The spinal fluid cell count in juvenile paresis compares very closely to the findings in acquired general paresis, and varies from no cells, to 310 cells per c.mm.

5. The most common finding in the colloidal gold curve is the typical "paretic" strong first zone curve, but other changes are found in from 10 to 15 per cent of cases; this is also comparable to the findings in adult neurosyphilis and in no way invalidates the clinical diagnosis.

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## BLOOD IODINE STUDIES\*

### V. THE BLOOD IODINE AFTER TOTAL THYROIDECTOMY IN MAN

GEORGE M. CURTIS, PH.D., M.D., LOUIS E. BARRON, A.B., M.D., AND  
FRANCIS J. PHILLIPS, A.B., COLUMBUS, OHIO

THE purpose of this paper is to present the results of a series of determinations of the blood iodine made previous to, immediately following, and subsequent to total thyroidectomy in man.

#### METHODS

Total ablation of the thyroid gland was made on five patients as a therapeutic measure for cardiovascular disease.<sup>1, 2, 3, 4, 5, 6, 7, 8</sup> All patients were maintained on the usual hospital diet, excluding only those foods known to have a high iodine content.<sup>9</sup> The usual amount of iodine in this diet, as determined in our laboratory, ranged between 0.161 and 0.207 mg. daily. Iodized salt is not used in our hospital diets. The patients were maintained at bed rest, and no iodine in any form was administered throughout their entire management. Their only daily source of iodine was in the food, water, and air intake.

Blood samples for the blood iodine determinations were drawn mornings in the postabsorptive state. The iodine determinations were made after the method of Phillips and Curtis,<sup>10</sup> which is an adaptation of the von Fellenberg procedure.<sup>11</sup>

During preoperative control periods, the blood iodine of the patients was determined. Following the establishment of adequate control determinations, total thyroidectomy was made. Three of the patients were subsequently hospitalized for the investigation of the late results. These investigations were made under conditions similar to those previous to thyroidectomy. Three of the patients have had blood iodine determinations made at frequent intervals since the total thyroidectomy.

Total thyroidectomy, in its strict literal sense, was not made upon the first two patients. Fragments of thyroid tissue, attached to the posterior capsule, in the adherent zone at the tracheo-esophageal sulcus, were purposely preserved in order better to protect the parathyroids and the recurrent laryngeal nerves. Into each of these fragments were placed numerous fine hemostatic ligatures. The total amount of thyroid tissue left behind in each of these patients was

\*From The Departments of Surgery and of Medical and Surgical Research, Ohio State University.

Received for publication, August 12, 1934.

Read before The American Physiological Society, March 30, 1934.

definitely less than one gram. In the last three patients, total thyroidectomy was performed. In none of the five patients was there any evidence of operative injury to the recurrent laryngeal nerves, or of subsequent parathyroid insufficiency. The latter was further checked by blood calcium and phosphorus determinations.

#### OBSERVATIONS

Curtis, Davis, and Phillips<sup>12</sup> found the normal blood iodine to range between 8.5 and 16.2 and to average about 12 gamma per cent. This is about the average finding as reported in the literature.<sup>13</sup>

The results of the present investigation are presented in summary form in Table I and Fig. 1. The initial blood iodine of all five patients, previous to extended hospital bed rest, was definitely increased over normal. The high-

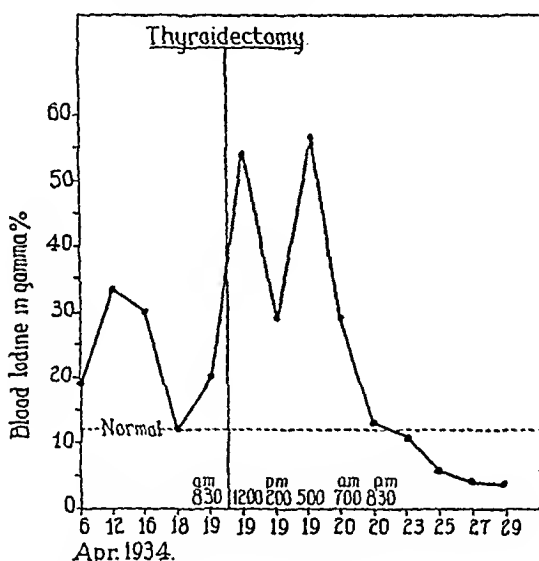


Fig. 1.—The blood iodine previous to, immediately after, and subsequent to total thyroidectomy in man (H. G., Case 5, Table I).

est value determined was 56 gamma per cent, the lowest, 19 gamma per cent. The average was 40 gamma per cent, Table I. When these patients were maintained on adequate bed rest, and on a diet low in iodine, the blood iodine level fell to normal in three patients just prior to thyroidectomy (Table I). In the fourth and fifth it remained elevated, 23.2 and 20.0 gamma per cent (Table I). The average immediate preoperative blood iodine was 14.6 gamma per cent (Table I). The reason for the persistent elevation in the two patients is not clear.

Several blood samples were obtained, in series, during the first twenty-four hours following thyroidectomy. In all five patients there ensued a transient but striking postoperative elevation of the blood iodine (Fig. 1 and Table I). This returned to normal in approximately twenty-four hours. Following this transient increase there ensued a gradual decrease in the blood iodine (Fig. 1).

The time required for this decrease to manifest itself varied among the patients (Table I). No satisfactory explanation, other than individual variation, can be offered for this lack of uniformity. In all patients, the blood iodine subsequently decreased to a level far below normal and remained at this low level. From the data presented, it appears that *following total thyroidectomy in man, the blood iodine is maintained at an abnormally low level.*

TABLE I  
THE BLOOD IODINE SUBSEQUENT TO TOTAL THYROIDECTOMY

SUBJECT	INITIAL BLOOD IODINE GAMMA %	PREOPERATIVE BLOOD IODINE GAMMA %	MAXIMUM IMMEDIATE POSTOP. BLOOD IODINE GAMMA %	EVENTUAL RESULTS GAMMA %
1. A. W.	55.5	13.5	25.1	2.0 94 days postoperative
2. E. K.	41.5	7.7	23.4	4.2 148 days postoperative
3. R. H.	56.0	23.2	35.0	5.3 18 days postoperative
4. C. R.	27.0	8.6	130.0	Sudden death, cerebral embolus, 40 hours postoperative
5. H. G.	19.0	20.0	57.0	4.1 10 days postoperative Fig. 1
Average	39.8	14.6	54.1	3.9

#### COMMENT

In 1922 Hudson<sup>14</sup> reported an increase of the blood iodine subsequent to total thyroidectomy in dogs. This occurred within the first forty-eight hours, and remained persistently elevated. Upon feeding sheep's thyroid gland, he reports a reduction of the blood iodine "so that it approximated the preoperative level." When thyroid feeding was discontinued, the blood iodine was again found to be increased.

Hudson's late results are questioned by Sturm,<sup>15</sup> who regards this persistent postoperative elevation of the blood iodine as due to blood analyses made during an inadequate postabsorptive period. The determinations, hence, were influenced by the alimentary absorption of iodine-containing foods. Sturm is unable to account for the decrease in the blood iodine level following the feeding of sheep's thyroid gland, since the latter is rich in iodine and, therefore, an increase rather than a decrease in the blood iodine level should have ensued. Our findings and conclusions are in accord with those of Sturm.

Associated with the immediate postoperative increase of the blood iodine in our patients, there was an increased excretion of iodine in the urine. This persisted for about two days and then subsequently returned to normal. The explanation for this is not entirely clear. It would at first seem that surgical manipulation and trauma to the thyroid gland might liberate iodine-containing substances into the blood stream, and thereby result in an increased loss of iodine in the urine after first increasing the blood iodine. The explanation is not as simple as this. Subsequent to partial thyroidectomy there ensues a great increase in the loss of iodine in the urine.<sup>16</sup> However, the urinary excretion of iodine is increased during the first forty-eight hours following other than thyroid surgery,<sup>17</sup> after which there ensues a gradual return to normal.

## CONCLUSIONS

1. Immediately following total thyroidectomy in man there ensues a transient increase in the blood iodine, which persists for about twenty-four hours.
2. After variable periods following total thyroidectomy, the blood iodine decreases to about one-third normal.
3. The presence of iodine in the blood following total thyroidectomy is probably due, in part, to the absorption of iodine from the alimentary tract.

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# THE VAN DEN BERGH REACTION (RING TEST TECHNIC) AND HEMOGLOBIN-BILIRUBIN INTERRELATION IN ICTERUS NEONATORUM\*

NORMAN W. ELTON, M.D., READING, PA.

THE findings presented herewith were obtained in a study of icterus neonatorum undertaken to determine the character of the van den Bergh reaction by the ring test technic and to correlate changes in blood hemoglobin and bilirubin levels during the first ten days of life. In twenty-four consecutive full-term infants, delivered in the Obstetrical Department of this Hospital, blood specimens were taken from the sagittal sinus through the anterior fontanel at frequent intervals during the neonatal period. Each infant was subjected to at least four fontanel taps for this purpose. On each specimen the icterus index, van den Bergh reaction, and bilirubin content of the serum were determined. In twelve infants, in addition to these tests, hemoglobin values by the improved Newcomer method, and erythrocyte counts were also determined. Including cord blood specimens obtained at delivery, 131 specimens have been examined. The data have been fully tabulated and will be discussed after a brief historical summary.

## HISTORICAL SUMMARY

All mammals exhibit a progressive increase in blood bilirubin content for a few days after birth.<sup>1</sup> In the human infant this physiologic icterus is usually visible in the skin or sclerae in about 60 per cent by the third day and disappears within a week. Opinion has long been divided as to the etiology of this icterus. Hoffmeier<sup>2</sup> originally believed it due to a sudden and rapid destruction of red cells, while Heynemann<sup>3</sup> later considered it primarily due to a disturbance of liver function. Between these two schools of thought, the division of opinion has persisted to the present time.

Van den Bergh and van Westrienen<sup>4</sup> were the first to note that serum from cord blood was always more yellow than that of the blood of the mother or of normal adults, regardless of whether or not the infants manifested a visible jaundice. They assumed correctly that the yellow color was caused by the pigment bilirubin.

Ada Hirsch,<sup>5</sup> using the diazo reaction of Ehrlich, which van den Bergh,<sup>6</sup> following Pröscher's suggestion, had just adapted to the detection and measurement of serum bilirubin, made quantitative determinations on the blood of the newborn. She found that during the first twelve hours of life the serum

\*From the Department of Pathology, St. Joseph's Hospital.

Received for publication, September 20, 1934.

Clinical material made available through the courtesy of Dr. Jackson Stuart Lawrance, Chief of Department of Obstetrics, St. Joseph's Hospital.

bilirubin rose rapidly, then more slowly, but progressively, to attain a maximum on the second or third day. Visibly jaundiced infants maintained this high level throughout the period of its visibility, while those who did not develop visible jaundice, or showed but a slight visible jaundice, exhibited the same sharp rise in the serum bilirubin curve, but a subsequent sharp drop to rather low values.

Van den Bergh concluded, from his own observations and those of Ada Hirsch, that the intensity of the hyperbilirubinemia per se bore no direct relationship to the subsequent development of visible jaundice in the newborn, and that no fixed threshold of visibility could be postulated in terms of quantitative bilirubin. This conclusion is fully sustained by the findings of the present study.

Herlitz<sup>7</sup> applied the bromsulphalein test for liver function in thirty infants not visibly jaundiced, and found a thirty-minute dye retention of 20 to 30 per cent in five under twenty-four hours of age, 10 to 30 per cent retention in seven under seven days of age, 20 to 30 per cent retention in five from two to three weeks of age, 10 per cent in eight from one to four months old, and no retention in five after the fifth month. In eight others, exhibiting a definitely visible icterus, the retention was no greater in corresponding age periods. His conclusion was that the liver obviously does not function properly in the newborn.

Considerations supporting excessive blood destruction as the underlying factor in icterus neonatorum are based on the decrease in the erythrocyte count from a condition of relative polycythemia on the first day of life to a count within normal limits ten days later, as occurs in the majority of infants, and the supposition that this polycythemia is induced by a relative anoxemia during intrauterine life, as suggested by Goldbloom and Gottlieb,<sup>8</sup> who found that guinea pigs subjected to a reduced atmospheric pressure developed polycythemia, and that on restoration to a normal environment the disappearance of this polycythemia was accompanied by a moderate increase in the serum bilirubin level.

Changes in the hemoglobin content and erythrocyte count in the blood of the newborn have been reported quite fully by Forkner,<sup>9</sup> whose average findings for the first, fourth, seventh, and tenth day periods were 20.49, 21.11, 19.46, and 19.04, respectively for hemoglobin values in grams per 100 c.c., and 6.0, 5.5, 5.6, and 5.3, respectively for the red cell counts in millions. Forkner's figures for the variations in the red count are closely paralleled by those reported by Lucas,<sup>10</sup> who found 5.5, 5.2, 5.1, and 4.1 millions for corresponding periods. Other investigators have reported counts ranging as high as 8 millions. The studies of Aitkin<sup>11</sup> clearly show that the polycythemia develops after birth, rather than being already established prior to birth, and attains its maximum within forty-eight hours. The tendency of the erythrocyte count to decline during the first ten days of life, exclusive of the first twenty-four to forty-eight hours, is definite enough when averages are considered, but individual cases often exhibit marked deviations from the average trend, as is demonstrable by the fact that in three of Forkner's nine infants on whom at



least three determinations were made, the maximum red counts were obtained during the third to eighth days of life. Hence, in a study of icterus neonatorum simultaneous determinations of changes in hemoglobin and bilirubin levels in individual cases are essential.

#### THE VAN DEN BERGH REACTION

*The Immediate Reaction.*—In this series the van den Bergh reaction has been determined by the ring test technic advocated by Magath.<sup>12</sup> A measured amount of clear serum (1 c.c.) is placed in a 15 c.c. (graduated) centrifuge tube with a tapering lower end. The tube is then slanted and 0.5 c.c. of Ehrlich's diazo reagent is carefully overlayed on the serum from a pipette. Positive reactions are indicated by the development within a few seconds of a red ring of azobilirubin at the contact zone between the two fluids. Most of these reactions are practically immediate in occurrence when bilirubinate, originating from the liver parenchyma as a conversion product of bilirubin, is present even in very small traces. After a minute has elapsed, if no reaction has been observed, the tube is carefully shaken to mix a portion of the serum with the reagent. Because of the taper of the tube, serum near the bottom will not be so readily disturbed as the upper portion of the serum, and the contact zone may be lowered to any desired level. Undisturbed serum below may now be compared with the overlying serum reagent mixture, thus affording a satisfactory control for the detection of color changes and their speed in the same tube. Occasionally a red ring will develop in a lowered contact zone, although it failed to appear when the reagent was originally overlayed on the serum. These reactions are also considered positives, due to their definite relationship to the immediate reactions as shown by serial studies, although they should be qualified as taking place after a measured delay period. Hence the van den Bergh reaction, by the ring test technic, has been recorded as positive, implying an immediate reaction, or positive after a stated interval of two minutes or less.

The ring test technic alone appears to make possible so frequently the detection of positive van den Bergh reactions in icterus neonatorum, for they are not usually demonstrable by the ordinary procedure. The degree of hyperbilirubinemia is evidently not the determining factor in their production (e.g., Infants 15 and 22).

The golden reaction is characterized by a definite and immediate diffusion of a golden yellow color upward from the serum into the diazo reagent overlying it. This type of reaction is frequently encountered in the blood of the newborn, and may occur with or without the simultaneous or later development of the positive (red) reaction.

*The Delayed Reaction.*—The sensitiveness of the ring test technic is strikingly demonstrated in icterus neonatorum, for if, after a red ring has developed, the serum and reagent are thoroughly mixed, even a delayed reaction fails to manifest itself. It was observed that in several specimens of cord blood, although the ring test was negative, an atypical reddish brown

TABLE I  
INDIVIDUAL CASE DATA

INFANT NUMBER AND SEX	DAY OF LIFE	ICTERUS INDEX	VAN DEN BERGH REACTION	QUANTITATIVE BILIRUBIN MG./100 CC.	HEMOGLOBIN GM./100 CC.	RED COUNT IN MILLIONS	DEGREE OF VISIBILITY OF JAUNDICE
1 M	Cord	15.0	Negative	0.9	-	-	-
	1	18.7	Negative	1.8	-	-	0
	2	25.0	Golden	3.1	-	-	0
	4	23.1	Golden	1.3	-	-	0
	6	15.0	Negative	0.8	-	-	0
	8	15.0	Negative	1.3	-	-	0
2 M	Cord	18.7	Golden	0.64	-	-	-
	4	60.0	Positive	6.5	-	-	++
	6	75.0	Positive	9.7	-	-	+++
	8	60.0	Positive	8.3	-	-	++
	10	60.0	Positive	8.4	-	-	+
3 F	Cord	21.4	Golden	0.66	-	-	0
	2	37.5	Golden	3.2	-	5.3	0
	4	60.0	Positive	5.9	-	-	++
	6	50.0	Positive	2.0	-	-	++
	8	50.0	Positive	6.5	-	-	++
	10	30.0	Golden	2.8	-	-	0
4 F	Cord	10.0	Negative	0.72	-	-	-
	2	25.0	Golden	1.7	-	-	0
	4	37.5	Pos. (1 min.)	3.7	-	-	0
	6	30.0	Golden	3.1	-	-	0
	9	15.0	Negative	0.3	-	3.7	0
5 M	2	21.4	Golden	2.3	-	-	0
	3	30.0	Golden	2.2	-	-	0
	5	30.0	Pos. (1 min.)	2.3	-	-	0
	7	21.4	Golden	1.1	-	-	0
	9	18.7	Negative	0.9	-	-	0
6 M	2	37.5	Positive	3.1	-	-	++
	3	50.0	Golden	3.3	-	-	++
	6	50.0	Positive	4.6	-	-	+
	8	25.0	Golden	2.7	-	-	+
	10	30.0	Golden	3.9	-	-	+
7 M	Cord	15.0	Negative	1.3	-	-	-
	2	30.0	Golden	1.6	-	-	0
	3	50.0	Golden	5.2	-	-	0
	5	60.0	Pos. (1 min.)	6.3	-	-	+
	7	37.5	Pos. (1 min.)	4.0	-	-	+
	9	25.0	Golden	2.8	-	-	+
	10	30.0	Golden	2.0	-	-	0
8 M	1	18.7	Golden	2.7	-	-	0
	2	37.5	Golden	4.4	-	-	+
	3	21.4	Positive	4.2	-	-	+
	4	30.0	Negative	1.0	-	-	+
	7	15.0	Negative	0.5	-	-	0
	9	15.0	Negative	0.4	-	-	0
9 F	Cord	11.6	Negative	0.75	-	-	-
	2	37.5	Golden	2.7	-	-	+
	3	37.5	Golden	5.0	-	-	+
	5	50.0	Golden	5.9	-	-	++
	7	25.0	Pos. (1 min.)	2.7	-	-	0
	8	15.0	Negative	1.3	-	-	0

TABLE I—CONT'D

INFANT NUMBER AND SEX	DAY OF LIFE	ICTERUS INDEX	VAN DEN BERGH REACTION	QUANTITATIVE BILIRUBIN MG./100 C.C.	HEMOGLOBIN GM./100 C.C.	RED COUNT IN MILLIONS	DEGREE OF VISIBILITY OF JAUNDICE
10 M	Cord	15.0	Negative	0.6	—	—	—
	2	30.0	Golden	2.3	—	4.4	0
	4	37.5	Golden	3.6	—	—	0
	6	37.5	Golden	2.1	—	—	0
	8	30.0	Golden	3.8	—	—	0
	10	21.4	Golden	2.3	—	—	0
11 F	Cord	12.5	Golden	1.2	—	—	—
	2	30.0	Golden	3.3	—	—	0
	3	30.0	Golden	2.5	—	—	0
	5	60.0	Golden	5.3	—	—	++
	6	50.0	Golden	4.5	—	—	++
	7	50.0	Golden	4.6	—	—	++
	9	50.0	Golden	4.5	—	—	+
12 F	Cord	15.0	Negative	0.7	—	—	—
	3	21.4	Golden	1.0	—	—	0
	5	15.0	Negative	0.75	—	—	0
	7	12.5	Negative	0.3	—	—	0
13 M	Cord	12.5	Negative	1.9	—	—	—
	1	21.4	Golden	2.7	12.63	4.3	0
	3	37.5	Golden	3.3	14.39	4.2	+
	5	30.0	Pos. (1 min.)	4.1	19.84	4.6	0
	7	18.7	Golden	1.5	15.50	4.4	0
	9	16.6	Golden	1.5	12.77	4.5	0
14 M	Cord	12.5	Negative	1.0	—	—	—
	1	21.4	Negative	1.7	15.43	4.2	0
	3	43.0	Golden	5.0	16.23	4.3	++
	5	50.0	Pos. (v. weak)	5.2	16.91	4.6	++
	7	50.0	Golden	5.0	15.78	4.4	+
	9	37.5	Positive	3.7	18.34	5.4	+
15 M	1	15.0	Negative	1.7	14.38	4.6	0
	3	25.0	Positive	2.6	16.68	4.5	0
	5	25.0	Golden	1.5	15.43	4.5	0
	7	21.4	Negative	3.4	17.36	4.4	0
	9	21.4	Negative	1.0	14.55	4.0	0
16 F	Cord	21.4	Negative	1.2	—	—	—
	3	43.0	Pos. (2 min.)	4.8	11.00	4.0	+++
	5	50.0	Positive	6.7	15.08	4.4	+++
	7	60.0	Positive	7.5	14.90	5.1	+++
	9	60.0	Positive	12.3	13.75	4.6	+++
17 M	Cord	16.6	Negative	1.0	—	—	—
	1	hem.	Negative	2.2	15.08	4.1	0
	3	43.0	Positive	5.2	15.60	4.0	0
	5	50.0	Positive	4.1	19.54	4.2	++
	7	30.0	Pos. (2 min.)	2.9	14.90	4.1	+
Twin of No. 18	9	21.4	Positive	1.4	15.25	4.0	0
	1	30.0	Golden	3.0	17.59	5.8	0
	3	37.5	Positive	4.0	17.59	5.4	0
	4	—	—	—	—	—	+
	5	50.0	Positive	4.3	15.43	5.5	++
	7	37.5	Pos. (2 min.)	4.3	13.75	4.5	+
	9	37.5	Positive	2.2	16.23	4.3	0

TABLE I—CONT'D

INFANT NUMBER AND SEX	DAY OF LIFE	ICTERUS INDEX	VAN DEN BERGH REACTION	QUANTITATIVE BILIRUBIN MG./100 C.C.	HEMOGLOBIN GM./100 C.C.	RED COUNT IN MILLIONS	DEGREE OF VISIBILITY OF JAUNDICE
19 F	2	75.0	Pos. (deepens)	7.3	21.40	5.0	+
	4	83.3	Golden	7.8	16.15	4.8	++
	6	60.0	Positive	5.6	18.53	4.5	+
	9	43.0	Positive	4.8	16.91	4.5	+
20 F	Cord	15.0	Negative	0.8	—	—	—
	2	30.0	Golden	3.5	15.43	4.9	0
	4	50.0	Positive	5.6	16.68	6.2	0
	5	—	—	—	—	—	++
	6	75.0	Positive	13.8	16.23	5.2	+++
	8	75.0	Positive	12.5	14.55	5.4	+++
	10	—	—	—	—	—	+
21 M	Cord	15.0	Negative	1.4	—	—	—
	1	37.5	Golden	3.3	16.46	5.0	0
	2	50.0	Golden	3.4	13.89	4.5	+++
	2	Died with intraadrenal hemorrhage					
22 F	Cord	8.8	Negative	1.35	—	—	—
	1	25.0	Golden	2.3	20.74	4.4	0
	3	50.0	Golden	9.6	18.64	4.9	0
	5	43.0	Golden	5.2	16.91	4.5	+++
	7	37.5	Golden	4.4	18.04	4.8	+
	9	30.0	Negative	3.3	17.36	4.5	0
	11	15.0	Negative	1.5	17.38	5.5	0
23 F	Cord	10.0	Negative	1.4	—	—	—
	3	15.0	Golden	2.1	10.03	4.2	0
	5	15.0	Negative	1.4	14.39	4.2	0
	7	15.0	Negative	0.9	16.91	4.3	0
	9	10.0	Negative	0.35	13.85	4.0	0
24 F	Cord	10.8	Negative	1.1	—	—	—
	1	16.6	Negative	2.2	17.59	5.1	0
	3	Hemolysis	—	3.8	18.34	4.5	0
	5	Hemolysis	—	2.4	19.54	4.3	+
	7	21.4	Golden	2.0	17.36	5.3	0
	9	18.7	Golden	1.3	17.59	4.8	0

## NOTES:

1. Infant 21 died on second day. Autopsy revealed massive hemorrhage in right adrenal. Ductus venosus patent. No bile retention in liver lobules microscopically. Slight trace of fresh bile in duodenum and upper jejunum. Meconium column low in ileum.

2. Bilirubin values are given in milligrams per 100 c.c. of serum by Thannhauser and Andersen technic.

color developed in the upper layer of serum reagent mixture when the contact zone was lowered. Possibly these reactions, definite but not strong, are pseudoreactions, for they were not found in specimens of sinus blood, and they may imply that some reactions of the delayed type are not always true azobilirubin reactions.

The greatest value of the ring test lies in the fact that many reactions, which would be termed delayed by the ordinary procedure in the conditions with which jaundice may be associated, are proved to be true positives, as has been demonstrated in a previous study,<sup>13</sup> and the incidence and impor-

TABLE IA  
SUPPLEMENTARY DATA

INFANT NUMBER	BIRTH POUNDS	WEIGHT OUNCES	PERCENTAGE WEIGHT LOSS	DAY OF APPEARANCE OF FIRST YELLOW STOOL
1	8	9	0%	2
2	6		5%	5
3	6	$\frac{1}{2}$	2%	4
4	6	9	3%	4
5	6	1	3%	6
6	5	10 $\frac{1}{2}$	6%	2
7	8		8%	4
8	9	5	2%	4
9	5	9	8%	3
10	6	1	9%	6
11	7	8	3%	4
12	6	1 $\frac{1}{2}$	3%	3
13	6	9	2%	6
14	8	2	3%	6
15	6		2%	3
16	7	1	4%	4
17	4	15	0%	6
18	5	1	2%	6
19	5	12	9%	4
20	6	12	5%	3
21	—	—	—	—
22	8	3	5%	8
23	7		2%	7
24	8	6	2%	5

tance of the delayed reaction is markedly reduced. Serial studies during the development and recession of jaundice in adults indicate that only delayed reactions with very short delay periods are of any practical significance, and that they are definitely associated with the positive reaction itself, originating from a functional or pathologic disturbance of the bilirubin excretion mechanism in the liver. Five minutes is regarded as long enough to wait for a delayed reaction of any clinical significance.

#### INTERPRETATION OF FINDINGS

Correlation of the intensity and duration of icterus neonatorum with birth weight, sex, percentage weight loss in the neonatal period, and the appearance of the first yellow stool has proved difficult if not impossible, and certainly occult. Percentage weight loss may be regarded as a fair criterion of dehydration, but infants exhibiting no loss, or only a slight loss, develop jaundice of no greater or less intensity than others showing maximum losses. The time of appearance of the first yellow stool, affording evidence of active bile excretion after birth, is governed not only by the initiation of postnatal liver activity, but also by the variable interval of time required for the expulsion of the meconium column, and is, therefore, of little significance.

The interpretation of the development of the positive van den Bergh reaction during the course of icterus neonatorum is essentially the same as in the icterus evolving from interstitial blood extravasations,<sup>13</sup> in that it is indicative of the passage of a pigment overload through the functioning lobules of the liver. As an integral phase of the excretion process for bilirubin, conversion of bilirubin to a bilirubinate occurs before the pigment is finally excreted

into the bile canaliculi, and leakage of the positive-reacting bilirubinate from the sensitive and readily congested central zone polygonal cells of the liver lobules into the liver lymphatics or sinusoids causes the appearance of the positive van den Bergh reaction in the general blood stream. In the icterus resulting from the absorption and excretion of a surplus of bilirubin derived from the disintegration of hemoglobin in interstitial blood extravasations, the positive van den Bergh reaction, when it develops, occurs near the peak of the bilirubin curve, and is followed by a recession of the jaundice. Many of the curves obtained in this study of icterus neonatorum exhibit a similar phenomenon.

The contention that icterus neonatorum is caused chiefly by excessive blood destruction is not supported by the interrelation of bilirubin, hemoglobin, and erythrocyte changes in individual cases, for fluctuations in red counts and hemoglobin values often occur quite independently of and disproportionately to bilirubin changes, and losses in hemoglobin or red cells are not accompanied by corresponding rises in bilirubin levels. On the contrary, the only correlation existing with any degree of constancy is that rises in hemoglobin accompany rises in bilirubin, and that the hemoglobin declines with the bilirubin. Correlations with red counts exhibit even greater disparities.

Factors involving the liver, however, afford reasonable etiologic possibilities. The period of the patency of the ductus venosus Arantii, which acts as an Eck fistula during the first few days of life, corresponds exactly with the

TABLE II

OCCURRENCE OF POSITIVE VAN DEN BERGH REACTIONS WITH RING TEST TECHNIC IN ICTERUS NEONATORUM

DAY OF LIFE	NEGATIVES	POSITIVES	PERCENTAGE OF POSITIVES
Cord	18	0	0%
1	10	0	0%
2	11	2	15%
3	11	5	31%
4	4	4	50%
5	7	7	50%
6	4	5	55%
7	10	5	33%
8	4	3	43%
9	10	5	33%
10	4	1	20%

TABLE III

AVERAGE ICTERUS INDEX, HEMOGLOBIN CONTENT, AND BILIRUBIN CONTENT OF BLOOD OF NEWBORN

DAYS OF LIFE	ICTERUS INDEX	HEMOGLOBIN (GM./100 C.C.)	BILIRUBIN (MG./100 C.C.)
Cord	14.2 (18)*	—	1.0 (18)
1-2	31.6 (23)	16.42 (11)	3.1 (24)
3-4	40.0 (23)	15.60 (11)	4.2 (24)
5-6	44.2 (23)	17.08 (11)	4.4 (23)
7-8	32.9 (22)	15.95 (10)	3.7 (22)
9-10	28.8 (21)	15.66 (10)	2.9 (21)

\*Figures in parentheses indicate number of specimens on which the average is based.

phenomenon of icterus neonatorum. Furthermore, the circulatory changes incident to birth should have a marked effect upon both the hemoglobin and the bilirubin metabolisms.

The hemoglobin metabolism will be affected by the interruption of the arterial blood supply through the umbilical vein from the placenta and the institution of oxygenation of the infant's blood in its own pulmonary circulatory system. Immediately following birth the ductus arteriosus shunts a large amount of the blood in the pulmonary artery directly back into the thoracic aorta, until it undergoes a process of obliteration not entirely completed for several weeks, before the pulmonary circulation becomes independent. A partial anoxemia, due to the fully functioning ductus arteriosus at the time of birth, would reasonably account for the rapid increase in erythrocytes during the first hours of life, creating a relative polycythemia as a compensatory mechanism, operative until the ductus arteriosus undergoes sufficient initial stenosis to establish a more compatible circulatory balance for the free-living child. During this period of readjustment from a parasitic life to an independent existence hemoglobin will be in demand, and the large iron reserve with which the newborn are endowed<sup>1</sup> will be utilized.

Liver function will to some extent be impaired by this transient partial anoxemia subsequent to birth, but the patent ductus venosus, lessening the blood supply of the liver by diverting the portal flow directly into the vena cava will greatly impair its efficiency as an excretory organ for bilirubin, and retention of the pigment in the blood stream will result. If, when the liver finally begins to excrete bilirubin effectively, after the closure of the ductus venosus, a sufficient proportion of its lobules become active and the pigment retention has not been too great, lobular overloading will not be sufficiently intense to cause leakage of bilirubinate, and positive van den Bergh reactions will not appear in the general circulation; but if bilirubin retention has been marked, or the liver does not commit to action an adequate number of its lobules, then positive reactions may develop from the leakage of bilirubinate from the overload-congested central zones of the functioning lobules.

Hence, the changes in hemoglobin and bilirubin content taking place in the blood of the newborn, appearing as two independent and only indirectly related phenomena, may logically be considered to be induced primarily by the patency of the ductus arteriosus and of the ductus venosus respectively.

#### SUMMARY

In twenty-four full-term infants 131 specimens of blood have been examined at frequent intervals during the first ten days of life to determine the character of the van den Bergh reaction by the ring test technic and to correlate blood bilirubin and hemoglobin changes during the course of icterus neonatorum.

The positive van den Bergh reaction was found to occur with a frequency of 36 per cent in all specimens during the second to tenth days, and to attain a maximum frequency of 55 per cent on the sixth day.

Since the most complete studies reported in the literature on variations in erythrocyte counts and hemoglobin values in the newborn show that a relative

or absolute polycythemia develops after birth, and usually within the first two days of life, and since during this same period the bilirubin level is rapidly rising in the blood, the postulation of excessive blood destruction as the underlying cause of icterus neonatorum does not appear reasonable. Partial anoxemia induced by the patency of the ductus arteriosus, and a disturbance of liver function induced by the patency of the ductus venosus Arantii provide rational explanations of the transient polycythemia and jaundice during the early neonatal period as two independent phenomena.

No correlation has been found in the data derived from this investigation supporting the assumption that blood destruction is a factor of any appreciable significance in the etiology of icterus neonatorum. The levels of bilirubin and hemoglobin in the blood tend to rise and fall together, although at times subject to erratic fluctuations. The patency and closure of the ductus venosus are presented as the logical factors in the etiology, intensity, duration, and termination of this icterus.

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# RHEUMATOID ARTHRITIS AND ITS TREATMENT BY GOLD SALTS\*

## THE RESULTS OF SIX YEARS' EXPERIENCE

JACQUES FORESTIER, M.D., PARIS, FRANCE

THOUGH the work of Mollgaard of Copenhagen on the action of gold salts in experimental tuberculosis in calves has not been checked by many authors, there is sufficient clinical evidence that in well chosen cases of human tuberculosis these compounds are of value to check at least temporarily the evolution of the disease.

Feldt has shown that in experimental infections in animals gold salts had no action if injected at the time of the inoculation of bacteria. On the contrary they can control these infections if injected some time after the inoculation.<sup>1</sup> These experiments suggest that if those compounds have no direct bactericidal effect, they are able to stimulate the defense reaction, once they have started in the infected subjects. It may account also for the fact that gold compounds seem to be more effective in subacute and chronic diseases, than in acute infections.

If gold salts are active in a chronic disease like human tuberculosis, why should they not be active in another chronic disease in which an important infectious factor seems to be present? If our hypothesis were true, it should soon be demonstrable that gold salts are active in true rheumatoid arthritis and in all forms of infective arthritis. This was the basis of our conception and the start of our use of gold salts in the treatment of arthritis. More than six years have elapsed, and our experience based on over 550 cases treated and observed during at least two or three years has proved conclusively the correctness of our opinion. Injections of gold salts when properly administered have given better results in the treatment of atrophic (rheumatoid) arthritis and of all forms of infective arthritis than any previous method employed in France; on the other hand, they have little action, if any, on the different forms of degenerative arthritis, osteoarthritis.

With these ideas in mind, I started in 1928 to select a certain number of severe forms of chronic polyarticular arthritis, for treatment by gold salts.<sup>2</sup> Following the procedure employed in the treatment of tuberculosis, I experienced some ill-effects due to over-dosage, such as stomatitis and erythema; but in spite of the high percentage of such incidents (25 per cent) I was gratified to observe, after two years' experience, that more than 60 per cent of my patients had greatly benefited from this treatment. In a few instances the clinical results were so surprisingly good that, for the first time, I got the impression that the

\*From the Hôpitaux de Paris and the Hôpital Thermal Reine-Hortense, Aix-les-Bains.

patients were not only free from symptoms, but practically cured.<sup>3</sup> A recent paper of G. Slot and P. M. Deville and others, of London, confirmed our work on this subject.<sup>4</sup> In Germany the problem has been approached with success by Umber<sup>5</sup> and Zimmer.<sup>6</sup>

The chemotherapeutic treatment of chronic diseases is no easy matter, because the physician cannot be content with a mere temporary improvement but desires to find a complete cure if such is available. Furthermore, spontaneous remissions, or, more rarely, spontaneous arrest of the disease may be attributed to treatment. This is why we cannot judge the action of any new therapeutic either in a short period of time, or with a few cases.

In checking the evolution of arthritis we cannot rely only upon clinical symptoms; they must not be disregarded, but we would like to have a specific test which could inform us as to the value of the actual treatment.

We can, however, say that even if the problem has not yet been entirely solved since we have not yet any specific reaction in connection with rheumatoid arthritis, like the Wassermann in syphilis, we have at least two reliable laboratory tests, which give us a fair measure of the activity of rheumatoid arthritis. These are the resorcin-flocculation test of Vernes and the sedimentation rate of the red corpuscles test of Facheus-Westergren. For the past four years we have been strongly advocating the use of these tests for arthritis in France, and they are now beginning to be commonly accepted. The value of the latter has been pointed out by some English and American authors (Race,<sup>7</sup> Buckley, Dawson, M. H., Sia, R. H. L., Boots, R. H.,<sup>8</sup> Rawls<sup>9</sup> and others).

*The Resorcin-Flocculation Test of Vernes.*—In 101 cases of inflammatory arthritis, mostly of the polyarticular rheumatoid form, we have found with Coste and Lacapere an average figure of 44.3. On the other hand, in 54 cases of osteoarthritis, the average figure has been 14.7 which is very similar to the average figure obtained in supposedly normal subjects.

*The Sedimentation Rate of the Blood Corpuscles*<sup>10</sup> which has been introduced in medical practice by Facheus and Westergren is easier, and gives the most help. The technic which is the simplest and the most reliable is that of Westergren. We express it usually by measuring the height of the supernatant plasma after one, two, and twenty-four hours. The first hour is the most important (Normal: 4-6 mm. in men, 6-8 mm. in women). In the past five years we have made with Gerbay several thousand determinations, and we believe that all the proposed improvements in the technic of the method, are fallacious; since it is a non-specific test, and only the comparative values in the same patient are interesting and not the absolute figures. Our findings have been very similar to those of Dawson, Sia, and Boots, and we can state that in almost every case of rheumatoid arthritis in process of evolution the sedimentation rate (S.R.) is permanently accelerated, very often over 30 and 50 mm., for the first hour.

But the prognostic value of the sedimentation rate is far greater than its diagnostic value. Our patients under treatment with gold have been tested almost every month, and at the beginning and at the end of every series of in-

jections. By this method, it is possible to draw a curve which represents better than any other data the process of evolution of the disease. After an experience of over four years the following statements can be made regarding the sedimentation rate. If the curve remains on a high level, even falling slightly, but with no real tendency to come back to normal figures, it means that the disease is progressing and, if the patient is being treated, it shows that the treatment is of little effect. If the curve, even after a period of being stationary at a high level, tends very progressively to come down and reach normal figures (under 10 mm.) in a few months, it means that the progress of the disease is being arrested and that the treatment is efficient.

It will not be possible, however, to consider that the patient is really clinically cured until a long time has elapsed after the cessation of treatment. The sedimentation rate must remain normal when recorded at regular intervals of three to six months.

If the curve, even after a long period of normal figures, has a tendency to rise up again, even slightly, and even with no clinical symptoms, it is almost

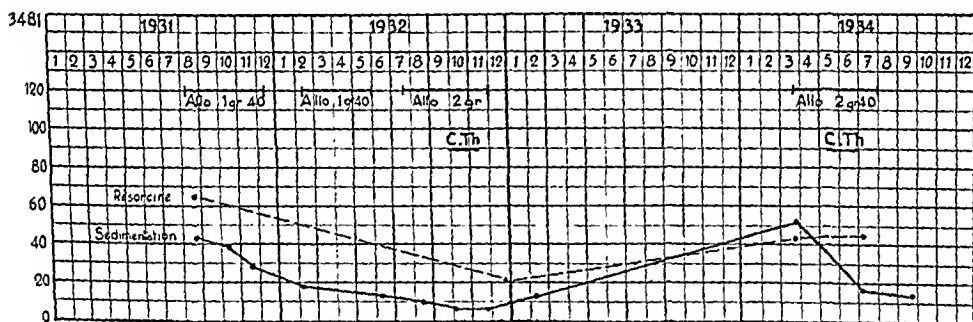


Fig. 1.—Graph of the sedimentation rate (first hour figures, Westergren's method) and resorcin flocculation, in a patient submitted to gold salt treatment. Case of generalized atrophic arthritis of eighteen months' duration, in a patient forty-two years of age, a mother of nine children, onset at eighth child's birth. Note slow descent of both curves under action of treatment, and relapse due to discontinuance of injections. Control of relapse was obtained by another series. Allo, gold salts. C. Th., thermal treatment.

certain that the disease is starting again and active treatment should be resumed at once (Fig. 1).

#### INITIATION OF THE TREATMENT OF GOLD SALTS

Before commencing a course of treatment by means of gold, cases should be carefully selected. A test of the tolerance of the patient toward the drug should be carried out, since, while it is effective, the method of treatment may at times give rise to untoward effects. The resorcin-flocculation test whenever feasible and the sedimentation rate must be made to test the actual condition of the disease and its tendency to aggravation.

#### INDICATIONS

The selection of cases for gold salts treatment is most important if disappointing results are to be avoided. Gold salts are active in every form of inflam-

matory arthritis, almost at every active period of its evolution, but may have practically no action in degenerative arthritis in its different forms. Here are the main indications:

I. True rheumatoid arthritis in its more serious forms at the beginning of its evolution (subacute) when swelling of the joints has just appeared; but even at much later stages, when distortion, ankylosis, and intense muscular atrophy have taken place. At middle age the form which occurs in women at the climacteric period can be equally treated with success.

We have seen considerable improvement amounting to cure in patients over eighty years of age. Multiple synovitis without articular involvement responds favorably to gold salts.

II. Chauffard-Still's disease in childhood.

III. Focal infection (metastatic) arthritis of the oligoarticular or monoarticular form, whatever the germ may be, gonococcus, *Bacillus coli*, dysenteric bacillus, etc., and also when the germ is unknown.

IV. Attenuated forms of tuberculous arthritis of the fibrous type, which may be held as transition types between true rheumatoid arthritis and what we call in France, "tumeur blanche." They are often associated with an enlargement of the lymph nodes, and respond satisfactorily to gold salts treatment, but generally need higher dosage. In true tuberculous arthritis, especially when visceral localization of the disease is coexistent, it is interesting to point out that gold salts treatment is not quite so successful, and higher dosage is required.

V. Ankylosing spondylitis which is an inflammatory arthritis of the small joints between the facets of the spine, resulting finally in the calcification of the perivertebral ligaments. This disease is associated with permanently accelerated sedimentation rate in its active stage. It responds favorably to gold salts treatment. Ankylosis can be prevented if the condition is early diagnosed and treated before general calcification of the spine takes place.

#### CONTRAINDICATIONS

Conversely, experience has shown that gold salts are of little, if any help, in the treatment of the different forms of osteoarthritis (arthrosis), namely, spondylitis deformans with true osteophytic productions on the bodies of the vertebrae, and hip joint arthritis of the form of *Mahm coxae senilis* (on the other hand, polyarticular arthritis of rheumatoid type involving the hip joints is a true indication for gold salts); osteoarthritis of the knee joints of old women, Heberden nodes, etc.; we do not use any more in such cases.

Once it is recognized that the patient is a case for gold salts treatment, the second question which arises is whether the organic resistance is satisfactory and will allow of such chemotherapeutic treatment. Severe diabetes, Bright's disease with marked albuminuria and tendency to uremia are, of course, definite contraindications. Hyperpiesia, except when very high, permits the use of gold

salts provided the treatment is well supervised. When a definite hepatic insufficiency is obvious, the treatment should be avoided, but when only mild symptoms of liver deficiency are detected, treatment is permissible. We do not treat patients who have shown definite hemorrhagic tendency (hemogenia, hemophilia). As a routine examination, in addition to the sedimentation rate, we carry out on our patients before the beginning of gold salts treatment the following laboratory tests:

Urine: albumin, sugar.

Blood: cholesterine, urica.

Blood corpuscles: complete count of white cells and red cells with Arneth's index.

Time of coagulation: time of bleeding.

If no contraindication is detected, the treatment may be commenced at once. If a definite focus of infection has been detected it will be attended to immediately, only if the general condition of the patient is favorable. If not, it seems wise to attempt to build up the patient's resistance, before submitting it to any surgical shock. The use of gold salts is a help in this matter.

#### CHOICE OF GOLD SALTS

The only active salts are those which contain a high proportion of gold (40 to 60 per cent) combined with a sulphur radical. Neither colloidal gold which acts as a colloid, nor gold chloride and cyanide are effective for our purpose.

As far as the form of injection is concerned, our method being an ambulatory one, we prefer the intramuscular route. Such injections may be given by skilled nurses or even by properly trained relatives of patients, provided the treatment is closely supervised by the physician.

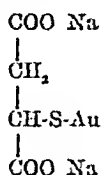
Gold salts can be injected either in aqueous solution or oil suspension. The compound which we used at the start of our experiments was an aurothiopropanol, sulphonate of sodium (allochrysine).

After three years' experience, we had arrived at the following method of administering allochrysine. Injections are given at weekly intervals, starting with doses of 5 cg. and repeated two or three times, the immediate reactions (focal reactions, temperature, malaise) being watched. If little or no reaction appears, weekly doses are raised to 10 cg. and the total for the first series ranges between 1.50 gm. and 2 gm. in polyarticular rheumatoid arthritis. When we wish to give a more intensive treatment, we diminish the intervals of injections to five or even three days, but we never increase the dose beyond 10 cg. for one injection.

The aurothiomalate of sodium (myochrysine)\* may be given in about the same dose as allochrysine, or even in higher doses. We generally use weekly injections of 10 to 20 cg. approximately. It is water-soluble and lipo-insoluble

\*Myochrysine is manufactured in U. S. A. by Merck & Co.

and is rather quickly absorbed either in aqueous solution or in oil suspension; it corresponds to the following formula:



The aurothioglycolate of calcium (myoral)\* is insoluble both in water and oil, therefore has to be injected in oil suspension only, so it is rather more slowly absorbed. It has given also favorable results.

The aurothioglucoase (solganol B) can be given in higher dosage than any of the gold salts previously mentioned. Starting with the same dose of 5 eg., we have been able to increase the weekly dosage up to 20 and sometimes 30 eg., the total amount for the series ranging between 2.50 gm. and 3 gm. The percentage of improvements is about the same as with the other salts.

Whatever compound is used, the interval between the first two series should not exceed six to eight weeks. Later on, it can be increased to three or four months, if the patient has been making good progress.

The dosages given are the results of experience varying between six years for aliochrysin and two or three years for the other salts. They have proved to be the best for obtaining a high proportion of good results with a small number of incidents and untoward symptoms. My colleague and coworker, F. Coste,<sup>11</sup> has recently proposed higher dosage in severe forms of arthritis, but I cannot recommend them as a routine method.

Recently, we have had the opportunity of using the original sanoerysin in oil suspension. It is well tolerated, but as solganol B it requires slightly higher dosage to give equal results to miochrysin, myoral, or aliochrysin.

In the light of our present experience we are not able to recommend the administration of gold salts by mouth, as an initial treatment in rheumatoid arthritis.

I. Whatever gold salt is chosen, our first principle is that treatment should be carried out by regular series of doses with intervals of rest as described, during at least one or two years, just as antisyphilitic treatment is carried out by chemotherapy with arsenic, mercury, or bismuth. *Any attempt to treat rheumatic patients by incomplete or by one series only, will lead to failure or rapid recurrence of symptoms after partial improvement.*

II. The second principle of the treatment of rheumatoid arthritis by gold salts is that it should be applied *as soon as possible* after the onset of the disease. Although the treatment is efficient even in cases of long standing, the results are far better when it is applied in early cases.

III. The third point is that the effect of the treatment should be checked at regular intervals by means of blood examinations. We have shown the im-

\*The aurothioglycolate is imported in U. S. A. by Fougera & Co.

portance of the resorcin-flocculation test and of the sedimentation rate. When the figures of these reactions decrease progressively it means that the treatment is effective, and it is unnecessary either to change the dosage or the particular gold compound in use. If, after two or three months' treatment the blood tests do not show any improvement in the biologic conditions, the physician will have either to increase the dosage for the next series of injections or even use another type of gold salt.

We have seen cases which did not respond to small doses greatly improved by injections of larger doses. We have seen a few cases where one type of gold salt was inactive and another successful. No very strict rules can be given on the particular point. It is a matter of clinical and laboratory experience which can only be determined for the individual by the physician. This is the part of gold treatment which really belongs to the art of medicine.

In conclusion, the dosage which we advocate is far smaller than that given to tuberculous patients by the majority of the profession.

In fact, arthritic patients are much more sensitive to injections of gold salts than tuberculous patients; perhaps this is because their allergic state is more marked. On the other hand, they respond most favorably to relatively small doses of gold salts. Whenever these doses are greatly increased, a high proportion of untoward incidents appear.

#### REACTIONS

The reactions to injections of gold salts may be local, focal or general. *Local* reactions are very rarely experienced, as injections of gold salts do not cause any irritation, when the preparation is properly dissolved in water or when suspended in oil. A short massage of the injected area is recommended after the injection to avoid nodules.

*Focal* reactions, revealed by pains in the joints and sometimes a temporary increase of the swelling, appear in about one-third of the cases, when small doses are used. They are generally more marked at the beginning of the series, and they tend to diminish and to disappear after 4 to 10 injections. They may be relieved by aspirin.

*General* reactions appear still more rarely when our method of small doses is accurately observed. They consist in most cases of a slight rise of temperature which does not exceed  $1^{\circ}$  F., they are rarely more marked. Such general reactions are desired by physicians using large doses of gold salts (K. Secher), but we prefer the small dosage method. Pruritus and urticaria are rather frequently met with, but provided they do not increase with each injection, they do not indicate the necessity for any change in the treatment. As with focal reactions, they tend to become less marked during the progress of the series. When they are severe, a reduction in the dosage of the following injection is indicated.

Another group of incidents which may be caused by gold salts are those which have been described by Milian under the neologism "Biotropisme." They consist of the appearance of the symptoms of some disease which was not previ-

ously suspected. Those most frequently met with are the symptoms described as "gold bronchitis" or "gold flu." Sometimes, they are more severe and may result in foci of congestion in the lung. Other forms of such accidents are represented by the appearance of boils. In four cases, we have observed herpes zoster.

All these accidents seem to be due to reactivation of a latent disease. They do not contraindicate the continuation of the treatment and generally disappear toward the end of the series.

Bronchial reactions are more frequently encountered with solganol B than with any other gold salt.

#### UNTOWARD SYMPTOMS

As is the case with the salts of all heavy metals gold salts may cause untoward symptoms which may affect three groups of organs:

1. Skin and mucous membranes
2. Liver and kidneys
3. Blood corpuscles

I. Reactions arising in connection with the skin are the most frequent, and may be large or small papules, surrounded by areas of erythema (with solganol B); erythema more or less generalized (with all other gold salts); in severe cases, which are very rare, dermatitis exfoliativa. Their intensity is generally in proportion to the doses injected. They may appear with the first or the last injections of a series. When they appear early and are only localized, they do not contraindicate continuation of the treatment, but in severe forms, injections must be at least temporarily stopped.

Reactions connected with the mucous membranes appear generally in the mouth, giving rise to small erosions, soreness and dysphagia; conjunctivitis, sometimes associated with keratitis has been observed. They also necessitate interruption of treatment. They are not commonly associated with skin troubles. In a few cases we have observed diarrhea which may be due to the effect on the mucous membrane of the bowels.

Since we have discarded large doses of gold in the treatment of arthritic patients, such skin and mucous membrane accidents have never been severe and the percentage has greatly diminished.

II. Albuminuria and tendency to renal insufficiency appear only in predisposed patients. As long as the functional value of the kidneys is good, and as no casts are detected in the urine treatment may be continued, even with moderate degree of albuminuria. With our small therapeutic doses, repeated series of injections have never pronounced any signs of nephritis in our patients.

III. We have observed in a very limited number of cases (4 out of over 500) reactions of agranulocytosis (2 cases) and purpura with hemorrhagic tendency (2 cases). Agranulocytosis has appeared in patients quite suddenly after several series of injections; this happened when we did not control the



use of amidopyrine in our patients. In our two cases, it was cured promptly by discontinuing the treatment and giving injections of sodium nucleinate.

Of the two cases of purpura, one was a mild one, because the treatment was immediately stopped, the other was very severe and was followed by fatality, owing to insufficient supervision of the treatment, gold salts and thorium X injections being given simultaneously by mistake.

Considering these accidents as a whole, they must not be taken too seriously, and it would be a great mistake to deprive arthritic patients of the benefit of gold salts, for fear of accidents which appear to be somewhat rare and in most cases are not serious.

It is most desirable that these accidents should be absolutely avoided, but such a desideratum has not yet become a reality. Sezary and Maurie have proposed to test the sensitization of the patients by skin tests made with various gold compounds.

To prevent skin reactions of severe intensity, German authors have suggested giving glucose, 40 to 80 gm. a day by mouth, during the whole of the series. In France, we use extensively total hepatic extract by mouth—10 grains, twice a day or better by intramuscular injections given with the gold salts. When such reactions have appeared, hepatic extract given by injection simultaneously with gold salts has enabled the treatment to be continued. We have used also with some success thiosulphate of sodium by intravenous injection and thiosulphate of magnesium by mouth or by intramuscular injection.

All the accidents described are considered generally rather due to intolerance, and sensitization appearing in some subject, than to real toxicity of the injected product.

#### CLINICAL RESULTS

All authors who have made use of injections of gold salts agree that in favorable cases the action of the treatment is most remarkable, on every kind of symptom. With our method of small doses, we must admit that the results obtained are rather slow and generally do not begin to appear before two or even three months, after the beginning of the injections. But, once they have appeared, they are progressive and continue to increase even after discontinuing temporarily the injections.

The first symptom to be definitely relieved is *pain*. If there was pain at rest, it disappears rapidly. If it existed only on motion, the movements become less and less painful and the patient is able to effect movements which had been impossible for months or years. We often judge of the amount of improvement experienced by the great diminution in the amount of aspirin or similar compounds necessary to enable the patient to lead a social life.

When patients are treated during the proliferative period, the second sign of improvement is the softening of the *swelling*, and later on its gradual disappearance.

The action of gold salts on the *general condition* of the patient is equally remarkable. While a certain number of cases of rheumatoid arthritis still con-

tinue to enjoy a satisfactory measure of health, many of them, especially during the period of evolution become pale and sallow, feel extremely depressed, and continually run a slight temperature. After two or three months of gold salt injections, the general strength is increased, appetite becomes better and the color improves greatly. In some cases an important gain of weight is obtained, but this rule is not without exception, as the restitution of the functions affords more exercise, some patients gain in flesh, and lose in fat. At the same time, the temperature curve, which is generally slightly above normal, tends very slowly but regularly to come back to normal and if the case be observed for several months, the action of the gold salt injections is quite obvious. The running of a normal temperature is one of the essential features which prove that the disease is arrested. The extent of the improvement observed depends largely upon the condition of the patient at the start of injections.

I. In relatively early cases, when the soft parts of the joints only are involved, which may last from one to three years in rheumatoid arthritis, the results of the gold salt injections can restore the functions almost to *restitutio ad integrum*. Here and there, may remain some partial stiffness and muscular wasting, but in most cases, patients in that stage of the disease may return to a normal life again, with a small amount of reeducation, massage, mechanotherapy, and sometimes spa treatment.

II. In cases of longer standing, when the cartilages have begun to be eroded, when muscular atrophy has become very severe and when chronic inflammation of the periarticular tissues has brought about fibrosis and retractions, the results of gold salts cannot be so satisfactory. They stop the inflammation, reduce the swelling and suppress the pain, but they are not able to rebuild the partially destroyed anatomical elements. Nevertheless, the action of gold salts in such cases is most remarkable, and it is surprising to observe how rapidly deformed joints and wasted muscles acquire a considerable amount of functional restoration provided the chronic inflammation which is characteristic of the disease has been checked.

Of course, in combination with injections of gold salts, and especially after they have begun to act, physiotherapy will play a great rôle in completing the restoration of the functions.

III. At the terminal stage of rheumatoid arthritis, at the period of distortions, bone destruction and permanent malposition of the limbs, one would think that chemotherapeutic injections would be of no help. Such was our belief at the start of our experience, but when we were begged by some miserable patients who had been in this condition for many years to give them a chance with the injections, we were greatly surprised to see that even in such desperate cases some partial but noticeable improvement could be obtained.

A young lady of thirty-five years, living in the south of France, who had been affected by polyarticular rheumatoid arthritis for thirteen years had developed fibrous ankylosis of both knees, one hip, both wrists, and one ankle. She was unable to walk more than a few steps even with crutches, and every attempt to correct her deformity was extremely painful. She was given two series of 1.50 gm. of allochrysine, and ten months later she was so

much benefited that she could walk with one stick and has been able to resume work. For the past three years, this improvement has been maintained by regular series of gold salt injections at rather wide intervals.

#### LABORATORY RESULTS

We do not believe that the conduct of gold salt treatment without any laboratory test is satisfactory. If clinical observation affords in some cases the possibility of judging the effectiveness of the treatment, in many cases it proves fallacious. Clinical observation alone is unable to decide whether chronic moderate swelling is due to an inflammatory process or to pure fibrous tissue, the equivalent of a scar. Conversely, we have observed a good many cases where no clinical symptoms of evolution were noticed but which showed a high sedimentation rate. When these patients are not treated, they regularly experience

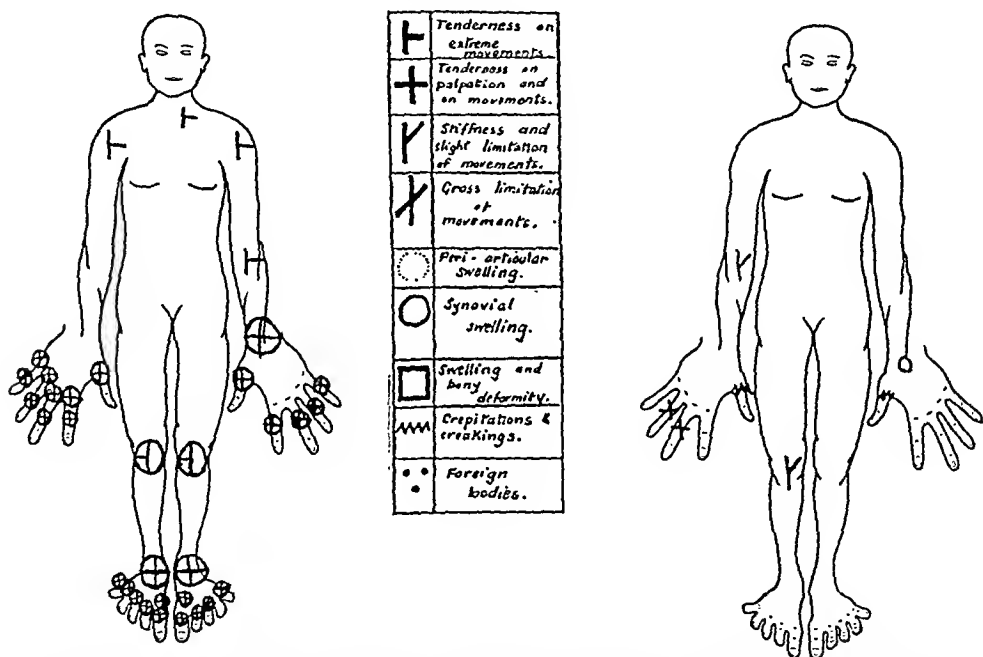


Fig. 2.—Diagram using Jansen's ciphers demonstrating joint changes before and after treatment. Case of polyarthritis of five years' duration in a man fifty-seven years of age. Note active swelling of numerous joints before treatment; and almost normal joints nine months later. Two series of 1.50 gm. of gold salts were given, with an interval of six weeks.

severe relapses within a short time, and it is to be pointed out that the action of gold salt injections after relapses is not so satisfactory as that originally obtained.

Last but not least is the question when the gold salt treatment has to be discontinued. None of our cases of polyarticular rheumatoid arthritis has ever been cured by a single series of injections; we never prescribe less than two series when we see a patient for the first time. As long as clinical symptoms of activity of the disease are observed, the duty of the physician is plain. He has to treat the patients by regular series at regular intervals. But when the patient shows no symptoms of activity of the disease, neither pain, swelling nor temperature, this is when the laboratory tests are of great importance. As long

as the patient shows an accelerated sedimentation rate above 10 or 15 mm. and an increase of the resorcin flocculation, above 30 or 40, we cannot consider the case as arrested, even if the patient is free from symptoms. He has to be treated, perhaps with smaller doses and at longer intervals, until these reactions become normal again. If a different attitude is adopted, sooner or later a relapse will appear, and as we have said relapses are more difficult to check.

Once the laboratory tests have returned to normal figures, is the treatment to be discontinued at once? We do not think so; we give a last additional series of gold salts which we call a "consolidation" series.

If both clinical and laboratory data are normal after this series, the patient is asked to come back regularly at three-month intervals during the next two years for a sedimentation rate test and resorcin-flocculation test. As long as the patient remains free from symptoms and these tests remain normal, no further injections are necessary. But if any of these tests show the slightest tendency to relapse, gold salt injections are resumed at once.

In order to have a basis of comparison for the joint changes of our patients during the course of the treatment, we have used for the past three years sketches designed by Hans Jansen of Copenhagen (Fig. 2). By means of a few very simple signs it is easy to describe accurately and quickly the changes in the joints for comparative studies, and once a sketch is completed, the physician understands at first glance the actual condition of the patient.

#### SUMMARY OF TOTAL RESULTS

Our experience in gold salt treatment is of six years' duration and deals with over 550 cases observed, about 400 at Aix-les-Bains and the remainder at Paris in the Cochin Hospital. We shall not give here any statistics as such statistics are ordinarily a matter of interpretation and may lead to mistakes, but we can state that between 70 and 80 per cent of our patients have responded favorably to the treatment. Among these, 50 per cent in recent cases and 20 per cent to 30 per cent in older cases of above two years' duration, have been apparently cured by 2 to 5 series of injections, and remained so in spite of discontinuation of any treatment, during the past two or three years. Such figures show the importance of early treatment.

In a general meeting of the "Ligne Française contre le Rhumatisme" in March, 1933, a discussion was opened on the method, and it was stated by all the physicians who have had the most experience in its use (Prof. Rathery, M.M., Hagueneau, Coste, Lacapère, Perles) that the results obtained were far better than with any medical method which they had used.<sup>12</sup> Dr. H. Forestier, my father, stated that in an experience of forty-three years' practice, he had not seen a single case of rheumatoid arthritis apparently cured during a period of several years. But since he had used gold salts, such cases were by no means uncommon.<sup>13</sup>

#### GOLD SALTS AND OTHER METHODS OF TREATMENT

Simultaneous use of gold salts and vaccine does not appear to be recommended, but alternate series may be of help in difficult cases. The use of

endocrine treatment as a complement, especially in middle-aged women, when thyroid and follicular extract may help the action of gold salts. The benefits of the different forms of physical treatment, which, in many countries, has been the only method applied to arthritic patients, are greatly improved by its combination with gold salt injections. All physicians of experience know that some forms of physiotherapy are deleterious to patients when applied at the acute stages of the disease. Once gold salts have checked such inflammatory evolution, these methods of treatment become possible and yield considerable benefit. Even orthopedic measures may be rendered possible by means of gold salt injections.

In fifteen very chronic cases of rheumatoid arthritis which had come to the stage of irreducible fibrous ankylosis of one or several joints: knees, hips, elbows; we have been able to reduce such bad positions by stretching the limbs under general anesthesia. Thanks to the preparatory treatment of gold injections, no inflammatory reactions have appeared as a result of this rather drastic procedure. Passive and active mobilization could be applied at once, reeducation in the thermal swimming pool at Aix-les-Bains, followed immediately; and we were glad enough to put on their feet after three, five, or even seven years, patients who would have remained bedridden for life.<sup>14</sup>

#### INTERPRETATION OF RESULTS

At the beginning of this article, we have stated that the use of gold salts in arthritis was the result of a conception of the latter disease being for the greatest part under the action of an infectious factor. We did not start our experience with the view that tuberculosis was a prominent factor in the etiology of rheumatoid arthritis; but in the past five years our belief has grown that such a factor has to be seriously considered in a certain proportion of cases. In addition to the clinical arguments presented thirty years ago by Poncet and Leriche, in their description of the "Rhumatisme Tuberculeux," some bacteriologic data have been collected in France in the past decade. With our co-workers<sup>15</sup> we have found two positive inoculations, and four dubious, with either blood, synovial fluid, or biopsy material collected from 21 patients showing the picture of general atrophic arthritis. These results point out the necessity of further research in this line. But even if the proof of the metastasis of Koch bacillus in joints is not given in atrophic arthritis, tuberculosis may act by bringing about joint allergy; and the combined influence of tuberculous allergy in the joints, could be the predisposing factor which initiates other infective germs (*streptococcus*) to fix themselves in the joints.

But gold salts have, in fact, no specific value against tuberculosis, they have been used with more or less success in various chronic infectious diseases. They seem specific of a certain type of tissue change which is called exudate in the lung, and proliferation in arthritis. Feldt has expressed the view that the action of gold salts could be ascribed to stimulation of the reticuloendothelial system.

## CONCLUSION

Six years' experience has demonstrated the action of gold salts on the evolution of chronic atrophic (rheumatoid) arthritis. Such treatment should be carried in selected cases over a long period by repeated series of injections, under strict medical supervision, with frequent blood controls, and in conjunction with an intelligent management of the general condition of the patients.

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## TRANSDUODENAL TREATMENT OF TAENIA SAGINATA INFESTATION\*

MEYER GOLOB, M.D., NEW YORK, N. Y.

I REPORT this case of taenia infestation not because of its interest per se, but for the importance it assumes in illustrating the new mode of therapy available to the general practitioner in such cases. One is naturally loath to publish conclusions drawn from a single case. But as I recall the spectacular recovery of the endoparasite, absolutely intact, during a single treatment, the delight of the patient in her quick relief—a vivid picture of becoming satisfaction as she views with amazement the erstwhile tenant to whom she played unwilling host; and as I contrast this with the unhappy history of our experience with conservative textbook methods—the repeated failures, prolonged infestations, marked anemias, and intensified general debility of the patient, then I am impelled to thrust aside such hesitancy. There is no room in my mind for the slightest doubt that here the old—the oral method—must give way to the new transduodenal treatment.

To Gunn<sup>1</sup> the treatment of saginata by the oral method has proved anything but satisfactory. Judging from the prolonged duration of the infestation in a number of his cases, one can hardly conclude otherwise. He remarks, rather quaintly, that on several occasions the medication produced such illness as to lead the patient to inquire if it would not be advisable to make a will! But of the tube treatment this author states “the results of this treatment are most gratifying to the patient as well as the physician.”

Schneider<sup>2</sup> reports a series of 87 cases of tape infestation treated via the duodenal tube, with 62 cures. Gunn reports 12 cases with the tube method, all successful, but ascribes the failure to recover the head of parasite in two of his cases to nonobservance of detail. It is quite true that the preparatory approach is of paramount importance in obtaining the best results.

### CASE REPORT

Female, aged twenty-eight, single. The symptomatology was of two years' duration, and was marked chiefly by a pronounced fatigue, “a very good appetite,” and loss of weight with obvious stigmas of asthenia. Altogether she presented a picture of a sickly human being “disgusted with doctors.” On each of several occasions when she received a vermifuge, there followed a passage of segments, the symptomatology persisting. The physical findings were negative, except for marked rings around the eyes and a facial pallor.

Laboratory Data: Blood cytology; hemoglobin 76 per cent (Sahli); erythrocytes 4,640,000; and leucocytes 15,000. *Differential Count*: Polynuclears 48 per cent, lymphocytes 42 per cent, monocytes 3 per cent, eosinophiles 6 per cent, and basophiles 1 per cent. *Smear*

\*Received for publication, September 28, 1934.

*Study:* Few pale red cells, few poikilocytes, occasional polychromatic erythrocytes. Eight days following treatment blood cytology revealed a normal white count (8,700) and no eosinophiles.

*Feces:* A few segments and very many ova of *Taenia saginata*.

*Directions to the Patient Preparatory to Treatment.*—

1. For two days take soft food and the third day, liquid only.
2. The bowels should be well moved by a laxative each evening.
3. Unless the bowels have moved freely take an enema.
4. Come to the office without breakfast. You may have a cup of black coffee on the morning of treatment.

*The emulsion consists of the following ingredients:*

Olei Aspidii	8 gm.
Mucilage Acacia	30 c.c.
Aqua	30 c.c.
MgSO <sub>4</sub> Sat. Sol.	30 c.c.

The mixture should be kept at body temperature. The added MgSO<sub>4</sub> to the solution is important to assure early catharsis, which in turn means less chance for absorption of the vermifuge.

*Record of the Procedure and Remarks*—

- 9:00 A.M. Levin tube swallowed.
- 9:55 Bile recovered, reaction alkaline.
- 10:00 Patient fluoroscoped. Although the bile was alkaline in reaction and the conventional tests showed a successful egress of the tube from the stomach into the duodenum, under fluoroscopic screen the distal portion of the tube was curved on itself, with its tip near the pylorus. With gentle pulling on the proximal part of tube, the other end straightened. Injection of the taenifuge at this time might have proved grossly disadvantageous, for the instilled emulsion of aspidium would have immediately regurgitated into the stomach.
- 10:35 Bile recovered was alkaline in reaction. Fluoroscopy disclosed the tip of the tube in the second portion of the duodenum, whereupon half the amount of the emulsion was slowly injected. The patient was perfectly calm and slept lightly. A syringe of hot water was injected to increase the probability that the parasite would be swept out, intact.
- 11:05 The remainder of the emulsion was injected. Ten minutes later the amount instilled was vomited. Vomitus saved. The duodenal tube was practically expelled and re-intubated. The stomach was washed out until the returned washings were clear and free from traces of the green emulsion. The tube was then fed to the patient until all tests verified the tube to be in the right position—in the second portion of the duodenum and to the right of the spinal column. Vomitus was kept warm and re-injected followed by 30 c.c. of a 33 per cent solution of magnesium sulphate.
- 11:10 Purgation began. Tube left in situ. The parasite intact, with head no larger than the head of a pin, was expelled. Tube removed.
- 11:30 Another bowel evacuation consisted of a brownish colored fluid. Patient felt comfortable and much relieved from the nausea that followed the vomiting. She stayed free from any of the toxic effects of drug absorption and displayed a marked joyful reaction, taking great interest in the examination of her endoparasite.

**NOTE:** To prevent gagging before injection of the taenifuge and immediately thereafter, it is important to clear the stomach of its mucus. This is especially desirable in the individual who salivates excessively. If purgation is delayed, it is important to leave the tube in position to permit the instillation of considerable quantities of hot water to hasten the purgative action and increase the likelihood of sweeping the parasite out in its entirety.



Notwithstanding all the precautions taken in this case, gagging and vomiting occurred. It therefore appears advisable to have extra medication should such accidents occur. Evidence of adequate preparation, as well as of cooperation on the part of the patient, is adduced from the fact that the discharge from the bowel consisted of only brownish colored water and the tape in its entirety.

The transduodenal expulsion of a parasite requires a smaller dose of a toxic drug, as the action on the parasite is direct. There is less chance for toxic absorption and the tape is usually expelled intact. Hospitalization is preferable but not necessary. With good care, the treatment may be given at an office equipped with a fluoroscope. The immediate proof of effective therapy is the exhibition of the head of the endo parasite in the expelled material.

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## WEIGHT REDUCTION WITH HIGH PROTEIN DIETS\*

LEO K. CAMPBELL, M.D., CHICAGO, ILL.

**D**URING these days of fads in reducing diets, many of which contain insufficient protein to maintain nitrogen equilibrium, physiologic accidents are not uncommon. A diet containing lower quantities of protein than that required to replace protein catabolism tends toward a lowered resistance, infections, degenerative lesions in vital tissues, and a fall in blood pressure. The study outlined in this paper makes use of diets which afford much more protein than is required for maintenance.

*Procedure:* The patients used in this study were from fourteen to sixty-seven years of age, and weighed from 133 pounds (60.0 kg.) to 317 pounds (143 kg.). The most obese patient was 185 pounds overweight and the nearest normal was 9 pounds overweight. All had normal basal metabolic rates with four exceptions, those being +13 per cent, -13 per cent, -17 per cent, and +20 per cent.

TABLE I

FOOD	TOTAL FOR DAY GRAMS	A.M.	NOON	P.M.	CARBO- HYDRATE	PROTEIN	FAT
5 per cent vegetables	600		300	300	18	6	
10 per cent fruits	100	100			10	1	
Egg	2	2				12	12
Lean meat	400		150	250		100	60
Butter	15	5	5	5			13
Bread	60	20	20	20	32	5	1
Total fuel values					60	124	86

\*From the Department of Medicine of Rush Medical College of the University of Chicago and Presbyterian Hospital.  
Received for publication, September 28, 1931.

TABLE II

PATIENT	AGE	SEX	INITIAL WEIGHT POUNDS	DIEET	DURATION OF PERIOD OF OBSERVATION	BASAL METABOLIC RATE	AVERAGE OF DAILY TOTAL URINARY NITROGEN IN EXPERIMENT	GRAMS AT END OF EXPERIMENT	AVERAGE OF DAILY TOTAL URINARY CREATININE IN EXPERIMENT	PHENOL/SULPHONEPH- TALINE TESTS AT END OF EXPERIMENT	NONPROTEIN NITROGEN OF BLOOD IN GRAMS AT END OF EXPERIMENT	BLOOD PRESSURE AT END OF EXPERIMENT	QUALITATIVE URINALYSIS AT END OF EXPERIMENT	Normal for age of patient	AVERAGE WEEKLY WEIGHT LOSS (POUNDS)
L. H.	27	F	200 ( 90.0 kg.)	CHO - P - F - Cal -	60 108 75 1347	+ 1%	16.0	1.49	89%	0.032	116/74	Normal for age of patient		1.4	
R. B.	36	F	325 (146.2 kg.)	CHO - P - F - Cal -	60 182 92 1596	- 6%	18.1	1.59	98%	0.023	118/70	Normal for age of patient		1.7	
P. G.	14	F	234 (109.3 kg.)	CHO - P - F - Cal -	60 105 75 1335	-13%	16.2	1.64	86%	0.032	116/76	Normal for age of patient		2.0	
L. G.	14	F	286 (128.7 kg.)	CHO - P - F - Cal -	127 101 94 1758	+ 4%	17.5	1.36	100%	0.038	124/78	Normal for age of patient		1.4	
D. H.	54	F	274 (123.3 kg.)	CHO - P - F - Cal -	60 132 92 1596	+20%	18.5	1.59	97%	0.035	140/80	Normal for age of patient		1.8	

TABLE II—CONT'D

PATIENT	AGE	SEX	INITIAL WEIGHT POUNDS	DIET	DURATION OF PERIOD OF OBSERVATION	BASAL METABOLIC RATE	AVERAGE OF DAILY TOTAL URINARY NITROGEN IN EXPERIMENT	GRAMS AT END OF EXPERIMENT	PHENOLSULPHONEPH- TALINE TESTS AT END OF EXPERIMENT	NONPROTEIN NITROGEN OF BLOOD IN GRAMS AT END OF EXPERIMENT	BLOOD PRESSURE AT END OF EXPERIMENT	QUALITATIVE URINALYSIS AT END OF EXPERIMENT	AVERAGE WEEKLY WEIGHT LOSS (POUNDS)
O. V.	67	M	217 ( 97.6 kg.)	CHO - 60 P - 132 F - 92 Cal - 1596	10 weeks	+ 5%	18.2	1.51	89%	0.037	134/84	Normal for age of patient	1.2
R. P.	35	F	163 ( 73.3 kg.)	CHO - 58 P - 112 F - 79 Cal - 1391	24 weeks	+2½%	18.8	1.40	93%	0.027	125/84	Normal for age of patient	1.4
O. P. M.	60	M	233 (104.8 kg.)	CHO - 60 P - 132 F - 92 Cal - 1596	20 weeks	-10%			47%	0.026	140/80	Normal for age of patient	2.2
W. B.	51	F	170 ( 76.5 kg.)	CHO - 62 P - 125 F - 85 Cal - 1495	6 weeks	-2½%	14.9	1.44	90%	0.030	120/70	Normal for age of patient	3.7
S. L.	29	F	135 ( 60.7 kg.)	CHO - 64 P - 115 F - 78 Cal - 1418	4 weeks	-17%			95%	0.035		Normal for age of patient	2.7

TABLE III

PATIENT	AGE	SEX	INITIAL WEIGHT POUNDS	DIET	DURATION OF PERIOD OF OBSERVATION	BASAL MET- ABOLIC RATE	AVERAGE WEEKLY WEIGHT LOSS (POUNDS)
P. R.	33	F	197 ( 88.6 kg.)	CHO - 60 P - 124 F - 86 Cal - 1510	14 weeks	+10%	1.2
A. F.	48	F	191 ( 85.9 kg.)	CHO - 64 P - 115 F - 78 Cal - 1418	11 weeks	+13%	1.4
H. G.	29	F	185 ( 83.2 kg.)	CHO - 58 P - 123 F - 87 Cal - 1501	8 weeks	+ 6%	2.1
K. F.	38	F	249 (112.0 kg.)	CHO - 49 P - 109 F - 77 Cal - 1325	13 weeks	+ 6%	1.9
R. W.	27	F	187 ( 84.1 kg.)	CHO - 64 P - 130 F - 72 Cal - 1304	10 weeks	-4½%	1.8
C. W.	54	F	200 ( 90.0 kg.)	CHO - 64 P - 140 F - 72 Cal - 1304	10 weeks	- 2%	1.9
G. C.	38	F	173 ( 77.8 kg.)	CHO - 58 P - 125 F - 97 Cal - 1605	6 weeks	+ 4%	2.3
E. C.	20	F	193 ( 86.8 kg.)	CHO - 64 P - 106 F - 75 Cal - 1355	11 weeks	-8½%	2.5
M. L.	38	F	185 ( 83.2 kg.)	CHO - 60 P - 124 F - 86 Cal - 1510	12 weeks	-4½%	1.8

None of these patients had demonstrable clinical evidence of pituitary or ovarian disease, and other than the four above mentioned exceptions there was no evidence of thyroid disease. All were of the common type of obesity; viz. that due to eating diets containing more calories than the daily calorie expenditure. There were twenty-seven patients in the group, twenty-one women and six men; one school-teacher, ten housewives, one nurse, two schoolgirls, two executives of manufacturing companies, two maids, one cashier, one clerk, two social workers, one traveling salesman, one librarian, one retired professor, one tailor, and one was unemployed. Each patient was either hospitalized or studied in the out-patient department for a period of ten days during each month, and during the initial study given a thorough training in quantitative dietetics. The diets provided 2 gm. of protein per kilogram normal body weight, a calorie value of from 20 per cent above to 39 per cent below basal maintenance, and fulfilled all

TABLE IV

PA-TIENT	AGE	SEX	INITIAL WEIGHT POUNDS	DIET	DURATION OF PERIOD OF OBSERVATION	AVERAGE WEEKLY WEIGHT LOSS (POUNDS)
G. K.	42	M	313 (142.6 kg.)	CHO - 59 P - 125 F - 86 Cal - 1510	6 weeks	6.0
J. D.	59	M	227 (102.1 kg.)	CHO - 60 P - 124 F - 86 Cal - 1510	8 weeks	3.0
P. F. G.	53	M	217 ( 97.6 kg.)	CHO - 60 P - 124 F - 86 Cal - 1510	4 weeks	5.0
B. S.	51	F	167 ( 75.1 kg.)	CHO - 60 P - 100 F - 72 Cal - 1288	6 weeks	2.5
N. S.	47	F	269 (121.0 kg.)	CHO - 43 P - 100 F - 78 Cal - 1274	4 weeks	3.2
E. B. S.	51	M	195 ( 87.7 kg.)	CHO - 60 P - 124 F - 86 Cal - 1510	6 weeks	5.0
R. L.	43	F	250 (113.8 kg.)	CHO - 60 P - 132 F - 96 Cal - 1596	7 weeks	2.2
M. C.	29	F	135 ( 60.7 kg.)	CHO - 62 P - 106 F - 76 Cal - 1356	6 weeks	2.0

requirements for normal metabolism in amino acids, salts, and vitamins. Four hundred grams of skimmed milk were incorporated in the diets of growing children. A sample diet containing 1,510 calories is shown in Table I.

During the period of hospitalization or study in the out-patient department the kidney function was observed by quantitative urinalysis, total nitrogen determinations on the urine by the Kjeldahl method, the phenolsulphonephthalein test, and the nonprotein nitrogen content of the blood. The blood pressure was recorded daily and the urine volume checked by total creatinine determinations, using the picramic acid method. Respiratory data were collected on patient L. H. Upon discharge after the period of study, each patient was instructed to return to his normal activities. Some, of course, took more exercise than others, but that was left entirely to the patient.

*Calorimetry:* The calorie expenditure measured by indirect calorimetry was determined on patient L. H. while on the high protein diet used for the weight reduction and also on an isocaloric diet, of minimum protein requirements but high in carbohydrate.

TABLE V A  
CALORIMETRY ON HIGH PROTEIN DIET

HOUR	DIET	CALORIES PER HOUR	R. Q.	URINARY NITROGEN	URINARY CREATININE
P.M.					
5:30	CHO - 20 P - 70 F - 42 Cal - 738				
6:00		71.6	0.79		
7:00		66.1	0.77		
8:00		69.8	0.76		
9:00		70.9	0.79		
10:00		71.7	0.79		
11:00		66.0	0.76		
12:00		69.8	0.83	2.8	0.33
A.M.					
1:00		64.6	0.78		
2:00		55.1	0.76		
3:00		48.3	0.79		
4:00		62.4	0.83		
5:00		59.5	0.77		
6:00		60.2	0.80	5.6	0.42
7:00		62.3	0.78		
7:30	CHO - 20 P - 15 F - 17 Cal - 293				
8:00		70.2	0.76		
9:00		66.0	0.81		
10:00		66.0	0.80		
11:00		63.2	0.79		
11:30	CHO - 20 P - 23 F - 16 Cal - 316				
NOON					
12:00		63.1	0.75	5.3	0.42
1:00		62.1	0.78		
2:00		65.9	0.79		
3:00		67.3	0.83		
4:00		64.2	0.76		
5:00		64.9	0.79		
6:00				3.8	0.41
Totals:	CHO - 60 P - 108 F - 75 Cal - 1347	1551.2		17.5	1.58

The caloric expenditure was 96 calories or 7 per cent more on the high protein diet than it was on the high carbohydrate diet. This increase in metabolism was probably due to the specific dynamic action of the protein.

#### SUMMARY

The weight of twenty-seven individuals having nonpathologic obesity was reduced during a period of from four to fifty weeks at an average rate of 1.2 to 6 pounds per week. The diets were of the high protein type containing approximately 2 grams of protein per kilogram ideal body weight. The caloric value of the diets was from 20 per cent above to 39 per cent below basal maintenance.

However most of the diets afforded a caloric value of about 20 per cent below basal requirements.

The patients maintained excellent health, continued all of their normal activities, and, of course, had increased energy as the weight loss progressed. The large amount of lean meat and vegetables prevented the hunger of which

TABLE V B  
CALORIMETRY ON HIGH CARBOHYDRATE DIET

HOURL	DIET	CALORIES PER HOUR	R. Q.	URINARY NITROGEN	URINARY CREATININE
P.M.					
5:30	CHO - 81 P - 24 F - 21 Cal - 609				
6:00		68.5	0.87		
7:00		62.3	0.90		
8:00		64.5	0.89		
9:00		61.6	0.84		
10:00		63.0	0.84		
11:00		63.7	0.76		
12:00		56.7	0.80	2.1	0.29
A.M.					
1:00		58.1	0.84		
2:00		57.4	0.84		
3:00		57.2	0.84		
4:00		54.3	0.81		
5:00		55.5	0.79		
6:00		58.6	0.88	1.2	0.06
7:00		58.0	0.85		
7:30	CHO - 62 P - 6 F - 12 Cal - 380				
8:00		72.3	0.93		
9:00		65.7	0.95		
10:00		60.0	0.84		
11:00		57.5	0.79		
11:30	CHO - 51 P - 8 F - 12 Cal - 344				
12:00		67.8	0.85	1.9	0.29
P.M.					
1:00		62.2	0.76		
2:00		63.0	0.83		
3:00		61.4	0.82		
4:00		58.2	0.80		
5:00		48.1	0.81		
6:00				1.6	0.13
Totals:	CHO - 194 P - 38 F - 45 Cal - 1333	1455.6		6.8	0.77

most individuals complain during weight reduction on low protein diets. There were no changes in the kidney function or blood pressure during any period of the observation. Nitrogen balance was maintained at all times. The caloric expenditure afforded by the high protein diet in the case of L. H. was 7 per cent above that of an isocaloric high carbohydrate diet.

## THE CONCENTRATION OF INDOXYL COMPOUNDS (INDICAN) IN BLOOD\*

HERMAN SHARLIT, M.D., NEW YORK, N. Y.

THE recent presentation by me<sup>1</sup> of a method for the quantitative estimation of indoxyl compounds (indican) in normal blood, on the use of reasonably small quantities thereof (2 c.c. plasma), opens anew the opportunity to study the clinical significance of varying concentrations of this heterocyclic nitrogen compound in human blood. Heretofore two technical difficulties prevented proper clinical studies of indican in blood; (1) the inability to measure the substance in small quantities of apparently normal blood and (2) the difficulty of securing pure indican for use as a standard for a satisfactory quantitative determination of indoxyl compounds. A more sensitive chromogenic reaction for indoxyl overcame the former; and the introduction of color filter colorimetry made cobalt sulphate solution a satisfactory color standard and so circumvented the latter difficulty.

A similar technic of mine<sup>2</sup> for the estimation of indican in urine, has disclosed daily excretions well in excess of what has been revealed by earlier methods; based on the Obermeier reaction, 11 mg. per day; on the use of Jolles' reaction, a maximum of 40 mg. and with this latter figure as a minimum, to even 300 mg. a day, as revealed by this newer method.

### METHOD FOR THE DETERMINATION OF INDOXYL COMPOUNDS IN BLOOD

The estimations are made on blood plasma, potassium oxalate or sodium citrate being used as an anticoagulant in the collection of the blood.

Reagent A: Protein coagulant, made by adding to each 25 gm. of trichloroacetic acid 100 c.c. of distilled water.

Reagent B: One per cent potassium persulphate in distilled water.

Reagent C: One per cent thymol in 95 per cent ethyl alcohol.

Reagent D: Acid reagent, made by adding to each 12.5 gm. of trichloroacetic acid 100 c.c. of concentrated hydrochloric acid (C.P.).

Reagent E: Glacial acetic acid.

*Procedure:* Place 2 c.c. of blood plasma in a test tube and dilute to 3 c.c. with distilled water. Add from a buret 5 c.c. of Reagent A after the following manner: three successive additions of 5 drops, each addition followed by vigorous stirring with a glass rod for fifteen seconds. This detail is most essential. It effects a fine and quite complete protein precipitation, resulting in a satisfactorily clear filtrate and maximum adsorption of the acid by the protein particles. The balance of Reagent A is added in two approximately equal portions with stirring after each. The tube is then centrifuged at moderately high speed for ten minutes. The filtrate is then delivered, through a 5.5 cm. quantitative filter paper, into a 15 c.c. centrifuge test tube graduated in 0.1 c.c.

\*From the Chemical Laboratory of Beth Israel Hospital.  
Received for publication, September 29, 1931.



To 6 c.c. of the filtrate are added successively 5 drops of Reagent B, 0.5 c.c. of Reagent C, and 5 c.c. of Reagent D. The tube is then momentarily corked and inverted and then allowed to stand at room temperature for a minimum of fifty minutes. Within five minutes after the last reagent is added, a dense white cloud forms in the mixture (ethyl trichloroacetate). In about fifteen minutes or sooner, if approximately 0.015 mg. of indican is present in the mixture, this cloud takes on an unmistakable purple tinge. When this occurs, it is necessary to resort to the use of the boiling water-bath completely to release all the indoxyl for a quantitative production of the pigment condensate (indoxyl-thymol compound). In this event, the tube is at any convenient time placed in a boiling water-bath and kept there for twelve minutes. If no distinct purple color develops within fifteen minutes, heating is unnecessary.

At the end of the fifty minutes at room temperature, or after the use of the water-bath, when needed, the tube is centrifuged at high speed for ten minutes, completely to throw down the ethyl trichloroacetate and the indoxyl-thymol compound contained therein. The volume of the ester formed is approximately 0.25 c.c. with the determination carried out at room temperature and about 0.6 c.c. when the water-bath is used. The supernatant fluid is then pipetted off, leaving a maximum of 0.3 c.c. of overlying watery acid mixture. This mixture of acetate and acid water is diluted to exactly 2 c.c. with Reagent E delivered from a buret. On stirring, a perfectly transparent colored solution results. Occasionally a fine white film is noted on the surface of the acetate but this too dissolves in Reagent E. The solution is now ready for reading in a microcolorimeter, through a green filter (Wrattan No. 74) inserted in the eyepiece, as described for the urine method. For a standard a 0.75 per cent solution of  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$  (Merck, C.P.) is used.

The method of calculation for blood indican is:

$$\text{Mg. indican per 100 c.c. blood plasma} = \frac{0.0043 \times \text{reading of standard (mm.)}}{\text{reading of unknown (mm.)}} \times \frac{100}{1.5} = 0.123$$

#### METHOD FOR THE DETERMINATION OF INDOXYL COMPOUNDS IN URINE

Reagent A: One per cent potassium persulphate in distilled water.

Reagent B: One per cent thymol in 95 per cent ethyl alcohol.

Reagent C: Acid reagent, made by adding to each 100 gm. of trichloroacetic acid 200 c.c. of water and 200 c.c. of concentrated hydrochloric acid.

Reagent D: Glacial acetic acid.

**Procedure:** Place 5 c.c. of urine in a volumetric flask and dilute to 100 c.c. with distilled water. To 2 c.c. of this dilution in a 15 c.c. centrifuge tube (graduated in 0.1 c.c.) are added 5 drops of Reagent A, 0.5 c.c. of Reagent B, and 5 c.c. of Reagent C. Before the addition of the acid reagent, the mixture is shaken for a second or two. The tube is immediately placed in a boiling water-bath and kept at that temperature for approximately five minutes. Before placing the tube in the water-bath, the mixture is slightly cloudy; it quickly clears at the temperature of the water-bath, soon followed by the formation of a second and more intense cloud. This is due to the formation of ethyl trichloroacetate which soon begins to settle to the bottom of the tube, carrying down with it a purple pigment (the indoxyl-thymol compound). The tube after removal from the water-bath is allowed to stand until it cools (about fifteen minutes), when all of the pigment-laden acetate has settled to the bottom of the tube, giving an acetate volume of approximately 0.5 c.c. The supernatant watery acid layer is then carefully removed with a pipette, leaving about 0.3 c.c. of watery layer above the colored acetate. This mixture (approximately 0.8 c.c.) of acetate and acid water is diluted to exactly 2 c.c. with glacial acetic acid, delivered from a buret. The mixture is stirred with a glass rod until it clears, giving a perfectly transparent, colored solution. This transparency is always achieved if, in pipetting, care is taken never to leave more than 0.3 c.c. of watery layer as overlying residue. The colored solution is now ready to be read.

The reading is done in the ordinary comparison colorimeter, with micro cups and appropriate plungers. In the eyepiece of the instrument is placed a green filter (Wrattan, No.

TABLE I

## BLOOD INDICANS IN NONSELECTED HOSPITALIZED CASES

CASE	MG. % NONPROTEIN NITROGEN	MG. % INDICAN	DIAGNOSIS
1	—	0.008*	Cerebellar vascular lesion; neurosis
2	—	0.040	Carcinoma of rectum
3	—	0.138	Secondary anemia
4	150	1.277	Prostatic hypertrophy (died)
5	120	5.877	Prostatic hypertrophy
6	35	0.163	Renal calculus
7	—	0.040	Hypertension
8	26	0.087	Pneumonia
9	37	—	Hemiplegia
10	33	—	Brain tumor
11	—	0.088	Pneumonia
12	33	—	Acute cholecystitis
13	30	—	Psychoneurosis; chronic ethmoiditis
14	26	0.106	Duodenal ulcer
15	54	0.329	Nephritis
16	40	0.068	Duodenal ulcer
17	37	0.073	Diabetes
18	30	0.009	Cardiac disease
19	37	0.044	Bronchopneumonia
20	54	0.057	Glomerular nephritis, with nephrotic manifestations
21	36	0.259	Pleurisy
22	29	0.073	Pneumonia
23	120	4.457	Prostatic hypertrophy
24	27	0.354	Hyperthyroidism
25	—	0.003	Graves' disease
26	25	0.155	Acute rheumatic fever
27	30	0.155	Nephritis and mastoiditis
28	30	0.130	Diabetes
29	43	0.130	Subacute glomerular nephritis
30	27	0.044	Coronary thrombosis
31	150	1.555	Bladder stone
32	50	0.384	Nephritis
33	40	0.270	Prostatic hypertrophy
34	60	0.207	Admitted for iridectomy
35	32	0.172	Auricular fibrillation
36	—	0.303	Cerebral embolism
37	37	0.171	Glaucoma
38	75	0.150	Nephritis
39	85	2.050	Cardiorenal disease
40	—	0.204	Renal calculus
41	54	0.143	Prostatic hypertrophy
42	40	0.016	Prostatic hypertrophy
43	40	0.163	Nephritis and ectopic pregnancy
44	85	0.450	Bladder stone
45	—	0.171	Umbilical hernia
46	35	0.122	Prostatic hypertrophy
47	30	0.020	Coronary disease
48	—	0.085	Diabetes
49	—	0.043	Inguinal hernia
50	33	0.213	Appendiceal abscess
51	54	0.106	Nephrectomy (calc ulus)
52	37	0.081	Hypertension, heart disease
53	100	0.524	Prostatic hypertrophy
54	85	0.259	Uremia
55	30	0.024	Psychoneurosis
56	30	0.030	Carcinoma of colon
57	40	0.087	Diabetes
58	—	0.050	Diabetes
59	35	0.293	Prostatic hypertrophy

TABLE I.—CONT'D

CASE	MG. % NONPROTEIN NITROGEN	MG. % INDICAN	DIAGNOSIS
60	46	0.106	Senile cataracts; detachment retina
61	50	0.641	Nephritis
62	35	0.065	Nephritis
63	32	0.130	Carcinoma of bladder
64	32	0.059	Prostatic hypertrophy
65	29	0.106	Ureteral calculus
66	46	0.163	Diabetes, cirrhosis of liver
67	200	2.457	Acute nephritis
68	54	0.188	Myoma of uterus
69	28	0.204	Pneumonia
70	35	0.352	Arthritis
71	—	0.106	Cataract
72	—	—	Diabetes
73	120	2.209	Acute nephritis
74	—	—	Diabetes
75	46	0.188	Nephritis
76	66	0.184	Bronchopneumonia
77	50	0.106	Hydronephrosis
78	33	—	Carcinoma of bladder
79	35	0.160	Arteriosclerosis
80	42	0.016	Acute sigmoiditis
81	42	—	Prostatic hypertrophy
82	30	0.032	Nephritis
83	40	—	Bronchopneumonia
84	31	—	Abscess of foot
85	29	0.147	Diabetes
86	33	0.224	Acute nephritis
87	—	0.032	Diabetes
88	30	—	Psychoneurosis
89	42	0.081	Diabetes
90	27	—	Diabetes
91	35	0.110	Nephritis
92	100	0.377	Intraabdominal tumor
93	54	0.188	Myoma of uterus
94	28	0.204	Pneumonia
95	66	0.057	Prostatic hypertrophy
96	37	0.089	Carcinoma of bladder
97	42	0.089	Arteriosclerosis
98	32	0.002	Neurosis
99	28	0.045	Diabetes
100	200	2.460	Acute nephritis

\*Normal indican measurements are set in black type.

- 74). For a standard solution we use a 1.5 per cent solution of  $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$  (Merck, C.P.).  
The method of calculation for urine indican is:

$$\text{Mg. indican per 0.1 c.c. urine} = \frac{0.0086 \times \text{reading of standard (mm.)}}{\text{reading of unknown (mm.)}} - 0.0009$$

Blood indican concentrations will require interpretation in the light of the following determined features of its excretion. Pure indican, parenterally or orally administered to rats, is rapidly and completely excreted in the urine.<sup>3</sup> Indican concentrations in the blood of normal persons (blood taken in the morning before breakfast) give 0.06 mg. per cent as an apparently upper limit, ranging down to immeasurably small concentrations. The latter findings are tabulated as "blank" in Table I.

Based on facts disclosed up to now, indican must be considered as of wholly exogenous origin, formed from absorbed indole by way of oxidation to

indoxyl and conjugation with a mineral acid, to the potassium salt of indoxyl sulphuric acid (indican). While a complete explanation of the synthesis of indican, this is but a meager and partial story of the metabolism of indole; this latter, however, does not concern us in this presentation. The point is that such indican as is formed from available indole is rapidly excreted by way of the kidney; and that under the conditions usually applied in taking blood samples for clinical studies (fasting stomach; sixteen hours after last meal) the indican concentration in normal blood approaches zero as a limit. It would thus appear that the interpretation of increased concentrations of blood indican would require emphasis on the failure of the kidneys to excrete rather than on the probability of excessive indican formation. To what extent this point of view is correct or how it must be modified in the light of more knowledge, awaits further study. Below are given indican estimations on the bloods of one hundred nonselected hospitalized patients, together with simultaneous estimations of the total nonprotein nitrogen in the blood of many of them.

There is no need to refer specifically to such publications as have demonstrated markedly increased concentrations of indican in the bloods of persons having nitrogen retention. Such findings were to be expected. What is to be stressed is study of the significance of small increments in concentration above the normal. And what is particularly to be noted in Table I is the extreme degree of retention that may be achieved, over 100 times the normal,\* which greatly exceeds the range of variation in other known metabolites. Furthermore, indican appears to be retained very early in hospitalized cases, only 33 per cent of this series of 100 nonselected cases showing normal figures. These facts suggest that further knowledge on the extent and circumstances of indican retention may provide a valuable diagnostic and therapeutic tool.

#### REFERENCES

1. Sharlit: A Method for the Quantitative Estimation of Indoxyl Compounds in Blood, *J. Biol. Chem.* 104: 115, 1934.
2. Sharlit: A Method for the Quantitative Estimation of Indoxyl Compounds in Urine, *J. Biol. Chem.* 99: 537, 1933.
3. Unpublished data of writer.

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\*In view of such high concentration of indican in uremia, it is possible to make an accurate measurement on a single drop of blood of such persons. And where, for purposes of differential diagnosis (coma, for example), a rapid and convenient test procedure is looked for, a single drop of blood taken from the finger may in fifteen minutes reveal the indications of a uremic blood picture.

## URINARY SULPHUR IN CHRONIC NONSPECIFIC ARTHRITIS\*

BEN D. SENTURIA, M.D., St. LOUIS, Mo.

AS EARLY as 1904, Goldthwait and his collaborators,<sup>1</sup> examining the metabolism of patients with chronic nonspecific arthritis, reported a definite loss of sulphur in two with atrophic (rheumatoid) arthritis and a pronounced sulphur retention in one with hypertrophic arthritis (osteoarthritis). Cawadias<sup>2</sup> claims "that the most constant metabolic disturbance met with in chronic rheumaties . . . is a negative sulphur balance indicating increased sulphur catabolism." He bases this conclusion on the finding of an increase in the total sulphur with a consequent decrease in the nitrogen-sulphur ratio in the urine of patients having either atrophic or hypertrophic arthritis. He also reports an increase in the urinary ethereal sulphates and neutral sulphur in a significant number of patients with chronic arthritis. Race,<sup>3</sup> on the other hand, denied the existence of a negative sulphur balance in these patients, but corroborated the occurrence in the urine of an increase in the neutral sulphur fraction of 20 patients in a series of 42 with rheumatoid arthritis. In view of recent reports<sup>4, 5, 6, 7</sup> concerning the use of colloidal sulphur parenterally administered as an adjunct in the treatment of chronic arthritis, and in view of the conflicting results of the fore-mentioned investigators and of the paucity of well controlled experimental data, we deemed it of sufficient interest to examine in adequately controlled experiments the alleged difference between healthy individuals and arthritic patients in regard to the partition of sulphur and the nitrogen-sulphur ratio in urine.

Our work comprised the determination of the sulphur content and its partition; namely, inorganic sulphates, ethereal, or conjugated sulphates and neutral, or unoxidized sulphur, in the twenty-four-hour quantities of urine of 18 patients with atrophic arthritis, of 41 with hypertrophic arthritis for comparison with the findings in those of 20 healthy individuals who corresponded closely to the pathologic cases both as to sex and age. Total urinary nitrogen was also determined for the purpose of observing the ratio of total nitrogen to total sulphur. All subjects were permitted to follow their customary dietary regime. In addition, we also hospitalized three patients with atrophic arthritis and three with hypertrophic arthritis and kept them for several days on a diet of known composition (Sherman's tables were used). It must be stressed that the collection of twenty-four-hour urine specimens without loss is a more difficult task than many experimenters realize. As a check we found it imperative to determine the creatinine on every specimen and to use for our purpose only specimens that were full twenty-four-hour collections beyond doubt,<sup>8</sup> since the analytical results

\*From the Laboratory and the Arthritis Division of the Out-Patient Department of the Jewish Hospital.

Received for publication, October 4, 1934.

Supported in part by a grant from The Doak Company, Cleveland, Ohio.

of fractions are worthless. The total nitrogen was determined by the micro-Kjeldahl technique; the total sulphur, inorganic sulphates and total sulphates by the Fiske benzidine method.<sup>9</sup>

Before discussing the results of our experiments, a brief consideration of the nature of sulphur metabolism may be in order. The total amount of sulphur in the urine depends on the amount and type of protein ingested and catabolized. According to Kahn and Goodridge<sup>10</sup> the sulphur content of proteins which occur in food varies between 0.3 and 2.2 per cent, with the consequence that the amount of sulphur eliminated in the urine as well as its ratio to the amount of nitrogen excreted may show wide variations, even if the amounts of nitrogen ingested and eliminated are quite similar. The total sulphur in the urine is distributed among three main groups of compounds; namely, inorganic sulphates, ethereal sulphates and neutral sulphur. Lewis<sup>11</sup> states that "the inorganic sulphate parallels the urea excretion and any factor that influences protein metabolism and elimination of urea will affect the excretion of inorganic sulphate." The ethereal sulphates are considered<sup>12</sup> in the main to be formed by the detoxification (conjugation with sulphuric acid) of indol and phenol derivatives produced by putrefactive bacteria in the intestines, a second part to be of endogenous origin<sup>11</sup> and a third to be derived from certain types of protein consumed.<sup>12</sup> The neutral sulphur consists of a heterogeneous group of unoxidized compounds considered to be mainly of endogenous origin, and, therefore, independent of the total amount of sulphur eliminated or of protein catabolized.<sup>12</sup> Folio<sup>12</sup> concluded that the distribution of the total sulphur in the urine among its three chief normal constituents depends on the absolute amount of total sulphur present. "With the reduction of the total nitrogen and total sulphur in the urine, we find a smaller percentage represented as inorganic sulphates and urea, and a relative increase in the other representatives of these two elements." Conversely, with an increase of the total sulphur in the urine, we find a greater percentage represented as inorganic sulphate, and a relative decrease in the ethereal sulphates and neutral sulphur.

TABLE I  
REPRESENTATIVE EXAMPLES OF THE RESULTS IN THE GROUP OF HEALTHY INDIVIDUALS

SEX	AGE	TOTAL* NITROGEN IN GM.	TOTAL SULPHUR AS S IN GM.	NITROGEN TO SULPHUR RATIO	INORGANIC SULPHATES AS S	ETHEREAL SULPHATES AS S	NEUTRAL SULPHUR AS S	PERCENTAGES			CREATININE	CREAT. COEFF.
								S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>		
F	54	5.8	0.529	10.1	0.408	0.062	0.059	77.1	11.8	11.1	0.949	14.1
F	20	6.4	0.690	9.3	0.530	0.037	0.123	76.8	5.4	17.8	1.286	26.9
F	35	7.3	0.691	10.7	0.507	0.109	0.075	73.4	15.8	10.8	1.314	22.0
M	65	8.8	0.749	11.8	0.592	0.087	0.070	79.0	11.7	9.3	0.845	18.4
F	34	9.2	0.789	11.7	0.640	0.071	0.078	81.1	9.0	9.9	0.908	19.3
F	28	9.9	0.683	14.5	0.528	0.074	0.081	77.3	10.8	11.9	0.962	17.4
M	44	10.5	0.755	13.9	0.606	0.115	0.034	80.3	15.2	4.5	1.543	21.5
M	51	11.4	0.772	14.8	0.707	0.014	0.051	90.3	3.1	6.6	1.992	26.6
M	35	11.8	0.885	13.8	0.653	0.058	0.144	76.4	7.7	16.9	1.380	21.4

\*The cases are listed on the basis of increasing levels of nitrogen excretion.

Our experimental results, presented in Tables I, II, and III, are in their general trend well in accord with the findings of Folin and Lewis. For the sake of economy in space, we do not include in Tables I, II, and III the complete series but only representative examples of the cases observed. In Tables IV and V, however, the average values were derived from the total number of subjects studied.

In all three groups of our subjects considerable variations are evident in the quantities of sulphur, both total and its divisions, and of total nitrogen as well as in the nitrogen-sulphur ratios. The average values for total sulphur,

TABLE II  
REPRESENTATIVE EXAMPLES OF THE RESULTS IN THE GROUP OF PATIENTS WITH ATROPHIC (RHEUMATOID) ARTHRITIS

SEX	AGE	TOTAL * NITROGEN IN GM.	TOTAL SULPHUR AS S IN GM.	NITROGEN TO SULPHUR RATIO	PERCENTAGES			CREATININE	CREAT. COEFF.			
					INORGANIC SULPHATES AS S	ETHEREAL SULPHATES AS S	NEUTRAL SULPHUR AS S					
					S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>					
F	45	5.2	0.383	13.6	0.305	0.027	0.078	72.6	7.0	20.4	1.618	28.4
F	38	5.8	0.510	11.4	0.399	0.090	0.031	78.2	17.7	4.1	0.790	19.5
F	28	6.7	0.559	11.9	0.426	0.061	0.072	76.2	10.9	12.9	0.843	16.8
F	19	7.4	0.602	12.3	0.424	0.077	0.101	70.4	12.8	16.8	1.027	16.9
F	20	8.3	0.598	13.9	0.482	0.080	0.036	80.6	13.3	6.1	0.930	18.3
F	50	9.2	0.619	14.9	0.499	0.052	0.068	80.5	8.5	11.0	1.053	16.2
F	30	10.0	0.691	14.5	0.562	0.062	0.067	81.3	9.0	9.7	1.092	16.5
F	35	11.9	0.902	13.2	0.807	0.069	0.026	89.5	7.6	2.9	1.403	20.1
F	39	15.4	1.400	11.0	1.267	0.100	0.033	90.5	7.1	2.4	1.453	14.7

\*The cases are listed on the basis of increasing levels of nitrogen excretion.

TABLE III  
REPRESENTATIVE EXAMPLES OF THE RESULTS IN THE GROUP OF PATIENTS WITH HYPERTROPHIC ARTHRITIS (OSTEOARTHRITIS)

SEX	AGE	TOTAL* NITROGEN IN GM.	TOTAL SULPHUR AS S IN GM.	NITROGEN TO SULPHUR RATIO	PERCENTAGES			CREATININE	CREAT. COEFF.			
					INORGANIC SULPHATES AS S	ETHEREAL SULPHATES AS S	NEUTRAL SULPHUR AS S					
					S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>		
F	54	5.9	0.410	14.2	0.288	0.015	0.107	70.3	3.6	26.1	0.980	15.2
F	50	6.3	0.766	8.2	0.543	0.067	0.156	70.9	8.6	20.5	0.868	17.7
F	33	7.2	0.627	11.5	0.511	0.068	0.048	81.5	10.8	7.7	0.837	18.2
F	45	8.6	1.239	6.9	1.061	0.097	0.081	85.6	7.8	6.6	1.444	18.0
M	58	9.4	0.744	12.2	0.628	0.059	0.087	81.0	7.7	11.3	1.222	19.8
M	57	10.6	0.939	11.3	0.766	0.107	0.066	81.6	11.4	7.0	1.364	20.0
F	43	11.5	1.120	10.3	0.925	0.067	0.128	82.6	6.0	11.4	1.750	24.4
F	52	12.4	1.063	11.7	0.901	0.048	0.114	84.9	4.4	10.7	1.270	15.5
M	44	13.3	0.937	14.2	0.797	0.032	0.108	85.1	3.4	11.5	1.460	22.0
M	45	14.0	1.083	12.9	0.895	0.111	0.077	82.6	10.3	7.1	1.490	26.9
F	35	15.5	1.225	12.7	1.034	0.092	0.099	84.4	7.5	8.1	1.423	13.7
M	61	17.8	1.247	14.4	1.012	0.133	0.102	81.2	10.6	8.2	1.758	23.9
F	65	18.4	1.259	14.6	1.133	0.039	0.087	89.9	3.2	6.9	0.940	12.2

\*The cases are listed on the basis of the following order: age, sex, total nitrogen, total sulphur, nitrogen to sulphur ratio, inorganic sulphates, ethereal sulphates, neutral sulphur, percentages, creatinine, creat. coeff.

\*The cases are listed on the basis of increasing levels of nitrogen excretion.

given in Table IV, are 0.705 gm., 0.736 gm., and 0.933 gm., respectively, for the healthy individuals, for the patients with atrophic arthritis and for those with hypertrophic arthritis. The inorganic sulphate fraction constitutes 79.7, 81.2, and 80.6 per cent of the total sulphur for the three groups of subjects in the order listed above; while the rest of the total sulphur is made up of approximately equal quantities of ethereal sulphates and of neutral sulphur. These results are in harmony with the views of Fiske regarding the total sulphur excretion and its partition of individuals on an ordinary average diet.<sup>13</sup>

While the average figures (Table IV) showing the percentage relationship of the three sulphur constituents to total sulphur are much the same for all three

TABLE IV  
EXTREME AND AVERAGE VALUES FOR SULPHUR AND ITS PARTITION

	TYPE OF CASE	NO. OF CASES	SULPHUR		AVERAGE	PROPORTION OF THE 3 TYPES OF S TO TOTAL S		AVERAGE
			LOWEST	HIGHEST		LOWEST	HIGHEST	
			gm.		gm.	per cent		per cent
Total Sulphur as S.	Normal	20	0.529	0.997	0.705			
	Atrophic arthritis	18	0.383	1.400	0.736			
	Hypertrophic arthritis	41	0.410	1.782	0.933			
Inorganic Sulphates as S.	Normal	20	0.415	0.826	0.562	71.1	90.9	79.7
	Atrophic arthritis	18	0.305	1.267	0.598	70.4	90.5	81.2
	Hypertrophic arthritis	41	0.288	1.678	0.752	70.3	89.9	80.6
Ethereal Sulphates as S.	Normal	20	0.014	0.134	0.070	2.5	16.9	10.0
	Atrophic arthritis	18	0.027	0.120	0.073	6.7	17.7	9.9
	Hypertrophic arthritis	41	0.015	0.232	0.090	3.2	19.6	9.7
Neutral Sulphur as S.	Normal	20	0.030	0.146	0.072	4.3	17.8	10.2
	Atrophic arthritis	18	0.026	0.102	0.066	2.4	20.4	9.0
	Hypertrophic arthritis	41	0.035	0.156	0.085	3.2	26.1	9.1

groups of our subjects, it may be noted that the averages of the absolute amounts of total sulphur, and, of course, of its three main constituents in the patients with hypertrophic arthritis are considerably higher than those in the rest of our subjects. The patients with atrophic arthritis, on the other hand, exhibit virtually the same sulphur excretions as the group of normal individuals. The high sulphur output in the group of patients with hypertrophic arthritis might suggest the conclusion at which Cawadias arrived, that these patients are losing sulphur, i.e., are in negative sulphur balance. At this juncture the determinations of the nitrogen excretion must be applied to ascertain the nitrogen-sulphur ratio as an indispensable control before the figures for the absolute sulphur excretions can be properly evaluated. As our analyses, summarized in Table V,

TABLE V  
EXTREME AND AVERAGE NITROGEN EXCRETIONS AND NITROGEN TO SULPHUR RATIOS

	NO. OF CASES	TOTAL NITROGEN IN 24 HOURS' URINE			NITROGEN TO SULPHUR RATIO		
		LOW-EST	HIGH-EST	AVERAGE	LOW-EST	HIGH-EST	AVERAGE
		gm.	gm.	gm.			
Normal	20	5.8	11.8	8.98	9.3	17.9	12.7
Atrophic arthritis	18	5.2	15.4	8.70	7.4	14.9	12.2
Hypertrophic arthritis	41	5.9	18.8	10.90	6.9	16.0	11.9



clearly reveal those patients who eliminated more sulphur also eliminated more nitrogen, i.e., 10.90 gm. as the average of nitrogen for the patients with hypertrophic arthritis as compared with the average of 8.98 gm. of nitrogen for the group of controls; the two values, increased nitrogen and increased sulphur, run parallel. This fact indicates that the increment in sulphur output is the result of an increased protein metabolism. In other words, the group with hypertrophic arthritis consumed on an average more protein, and most likely more food in general than did both the normal subjects and the group with atrophic arthritis examined in our experiments.

In addition, even when considering the average absolute values and the range of values for the ethereal sulphates and neutral sulphur fractions (Table IV), we find no significant differences between the normal persons and the two groups of patients with arthritis. The quantities of these two constituents in the patients with atrophic arthritis are virtually identical with those in the controls; for ethereal sulphates the figures are 0.073 gm. for the former and 0.070 gm. for the latter, while for neutral sulphur the values are 0.066 gm. and 0.072 gm., respectively. There is, likewise, practically a coincidence between the absolute values for neutral sulphur in the patients with hypertrophic arthritis and in the normal subjects. The range of values are 0.035 gm. to 0.156 gm. in the former and 0.030 gm. to 0.146 gm. in the latter, with average values of 0.085 gm. and 0.072 gm., respectively. Among the absolute values for the ethereal sulphate fraction in the patients with hypertrophic arthritis there are four instances with readings of 0.159, 0.162, 0.179, and 0.232 gm. which are definitely above the highest figure of 0.134 gm. found for the ethereal sulphates in the group of controls. Yet, the percentage relationship of ethereal sulphates to total sulphur in these four exceptional cases (13.7, 15.3, 19.0, and 19.6 per cent) are either within or insignificantly above the highest relative value of 16.9 per cent found in the healthy subjects. Consequently, no importance is attached to the average absolute value of 0.090 gm. for ethereal sulphates found in the group of patients with hypertrophic arthritis as compared with the average absolute value of 0.070 gm. found in the healthy class. Furthermore, the relative values for these amounts, namely 9.7 per cent and 10.0 per cent, respectively, warrant the conclusion that the difference in the average absolute values for the ethereal sulphates in the group with hypertrophic arthritis and the healthy group merely reflects the difference in their total sulphur excretions.

Similarly, as pointed out previously, the relationship between the nitrogen and sulphur elimination as expressed by the nitrogen-sulphur ratio is practically the same in all three groups of our subjects; 12.7 for the group of normal individuals, 12.2 for the group with atrophic arthritis and 11.9 for the group with hypertrophic arthritis (Table V). The range of values for this ratio of 9.3 to 17.9 found in our controls corresponds closely with the values of 13 and 16 established by Lewis<sup>11</sup> and that of 9.3 found by von Wendt.<sup>14</sup> Our average value of 12.7 practically coincides with that calculated from the total nitrogen and total sulphur reported by Folin<sup>15</sup> in the urine of six "normal" individuals. The average N:S value of 11.9 found in the class with hypertrophic arthritis is not considered to be significantly lower than the average N:S value of 12.7

determined in the normal subjects. This finding is decidedly contrary to the assumption of a negative sulphur balance in these patients. In only one of the 18 patients with atrophic arthritis and in four of the 41 with hyperarthritis were nitrogen-sulphur ratios observed below that of our lowest normal value of 9.3. The figures for these exceptional cases are 7.4 for the one patient in the former group, and 6.9, 6.9, 6.9, and 8.2 for the four patients in the latter group. We believe it would be too far-fetched to attribute special significance to such a relatively small number of exceptions.

As a further check on the sulphur balance of patients with chronic non-specific arthritis, the nitrogen to sulphur ratios in the food were compared with those in the urine of three patients with atrophic arthritis and three with hypertrophic arthritis who were hospitalized and whose food intake was accurately measured. The nitrogen and sulphur in the stool does not materially affect the findings in the urine and can be safely disregarded.<sup>16</sup> The results of this experiment are shown in Table VI.

TABLE VI  
NITROGEN TO SULPHUR RATIO IN FOOD AND URINE

	CASE NO.		N:S RATIO OF FOOD	N:S RATIO OF URINE
Atrophic arthritis	13	1st day	12.2	14.6
		2nd day	12.7	13.7
		3rd day	11.8	13.7
Atrophic arthritis	14	1st day	13.1	13.7
		2nd day	13.8	14.3
Atrophic arthritis	15	1st day	12.6	13.6
		2nd day	14.2	14.8
		3rd day	12.7	13.3
Hypertrophic arthritis	5	1st day	12.2	14.7
		2nd day	11.9	15.8
		3rd day	11.7	13.3
Hypertrophic arthritis	8	1st day	11.8	14.2
		2nd day	13.1	15.3
Hypertrophic arthritis	25	1st day	11.8	15.5
		2nd day	11.5	15.4

In no instance did the nitrogen to sulphur ratio in the urine become less than that in the food consumed during the corresponding twenty-four hours, thus illustrating that there was no loss of sulphur in either type of patient. The subjects in this series had not been under sulphur therapy previous to and during the experiment. As a matter of fact, our cases show a sulphur retention rather than a negative sulphur balance.

#### CONCLUSION

In our studies, the sulphur excretion and sulphur partition in the twenty-four-hour quantities of urine of 18 patients with atrophic arthritis and 41 with hypertrophic arthritis showed no appreciable deviation from those of 20 healthy individuals. In only one patient of the 18 with atrophic arthritis and in four of the 41 with hypertrophic arthritis were nitrogen to sulphur ratios observed

which were below the lowest value found in the normal subjects. On the whole our experiments disprove the alleged existence of an abnormal sulphur elimination or sulphur partition in the urine of patients with chronic nonspecific arthritis.

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# LABORATORY METHODS

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## STUDIES IN BACTERIOPHAGE II<sup>2</sup>

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### METHODS OF MAINTAINING AND TESTING THE POTENCY OF BACTERIOPHAGE

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HELEN ZAYTZEFF-JERN, M.D., AND FRANK L. MELENEX, M.D., NEW YORK, N. Y.

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#### A. INTRODUCTION

OVER two years ago we began to use bacteriophage in the Surgical Department of the Vanderbilt Clinic in the treatment of staphylococcus infections. The bacteriophage used at the beginning was prepared periodically in a laboratory having had a long experience in the preparation of phage and was sent to us in batches as needed.

The first two batches were apparently successful in shortening the duration of most of the lesions treated, and lysis of the strains in the test tube occurred regularly. However, on the arrival of the third batch the results suddenly became poor, and simultaneously the phage failed with many strains to produce complete lysis in the test tube. When we reported this it was said that in some way or other this phage must have lost its potency during the final heating process (60° for one hour on three successive days). The use of one of the next batches, however, met with varying success. On inquiring if there were any changes in the method of preparation of the different batches of phages which might have affected the potency, it was learned that in the last one, the phage was propagated at the expense of only one strain of staphylococcus, while for the preparation of phage of the first and second batches ten to twelve strains of staphylococci were used.

The same failure occurred when we produced our own phage by the method used by Dr. MacNeal of the Post Graduate Hospital, New York, who supplied us with five different strains of polyvalent staphylococcus phage and a stock culture upon which he propagated them. The five phages were propagated separately in plain broth at a pH of 7.6 to 7.8, 0.1 c.c. of a twenty-four-hour bacterial culture and 0.5 c.c. of phage were added to 10 c.c. of the broth. The tubes were incubated at 37° C. overnight and, if found to be clear, they were filtered and pooled before being used in the clinic.

The results at the beginning were encouraging. However, after a short period of success we were suddenly faced with a series of failures. Simultaneously it was found that the bacteriophage was beginning to lose its power to

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<sup>2</sup>From the Bacteriological Research Laboratory of the Department of Surgery, College of Physicians and Surgeons, Columbia University.

Received for publication, October 5, 1934.

produce complete lysis *in vitro*. The phage was then tested against old strains which it had formerly lysed, and it was found to be impotent.

Not recognizing the cause of this deterioration we made an immediate shift to strain BL obtained from Dr. Gratia then of the University of Brussels. This was found to be effective not only in the laboratory but in the clinic, and its period of success lasted for about two months only to be followed by a series of poor results. This time, however, the laboratory tests still showed a complete visible lysis of most of the strains of staphylococci, even to the eighth decimal dilution of phage, which d'Herelle<sup>1</sup> considers to indicate a fairly high degree of potency.

It was obvious that the potency of the bacteriophages which we were using was not constant, and it seemed to us to be important to find some method of insuring the potency of the phage. From a clinical point of view the term "potency" might be applied to the ability of the phage to arrest the course of the infection in the patient but from a biologic viewpoint, it applies to the ability of the phage to destroy and prevent the growth of the bacteria in the test tube. For this bactericidal or bacteriostatic property the word "virulence" has been used but this does not seem to be a suitable term for clinical potency. As yet no standard method of phage preparation has been recognized although various methods have been proposed. What are the factors which might be involved? We decided to investigate certain of these factors, for example the media employed, the bacterial strain used for propagating the phage, the concentration of the bacterial inoculum, the amount of phage used, the optimum temperature, and the method of testing for potency.

#### B. CULTURE MEDIUM

For a propagating medium some authors have recommended plain meat infusion broth; but MacNeal<sup>2</sup> has advised asparagin for intravenous injection to minimize the protein content while others, including d'Herelle and his followers<sup>4</sup> favor "savita" medium. We have employed all three in this study. We have not used the papain digest broth recommended by Asheshov.<sup>3</sup> We found that asparagin was not sufficiently nutritive for the development of either bacteria or bacteriophage. Staphylococci grew in it very slowly, yielding about 500 to 750 million organisms per c.c. after twenty-four hours of incubation at 37° C., and still less growth at a temperature of 32° C., or lower. This was, therefore, abandoned and replaced by "savita" medium used in d'Herelle's laboratory in Paris and by some of his followers. This is made out of 1 per cent "savita" extract, 0.5 per cent of sodium chloride, and adjusted to a pH of 8.1, which after boiling and autoclaving becomes 7.5 to 7.8.

A comparative study of staph-phage preparations in meat infusion broth and "savita" medium showed that the lysis of bacteria in broth is accomplished in a shorter period of time than in "savita." On a number of occasions, it was noticed that after eighteen hours of incubation at 32° C., the broth tubes were completely clear while "savita" was still slightly turbid. After twenty-four hours they usually became clear when left at room temperature. "Savita" phage always had more of a tendency to form secondary culture than broth phage. It was

also found that larger numbers of staphylococci could be completely lysed in broth than in "savita." Up to 700 million per c.c. could be completely lysed in broth but this number could never be dissolved in "savita" medium.

When measuring the number of bacteriophage corpuscles by the plaque count, we found that the count in broth phage was always somewhat higher than in "savita" phage under identical conditions. With respect to retaining the potency as measured by plaque count and lytic action in broth we found that broth phage retained it for a longer period of time than "savita" bacteriophage.

On the other hand, in our clinical application of bacteriophage for the treatment of staphylococcus infections, such as furuncles and carbuncles, in which bacteriophage was injected directly into the lesion, we found that broth phage occasionally gave a severe local reaction, while this never occurred with "savita" bacteriophage. Furthermore, when injected subcutaneously, "savita" phage gave practically no reaction while the area injected with broth phage was sometimes surrounded by a large zone of redness.

#### C. THE OPTIMUM TEMPERATURE FOR PROPAGATION

There has been no consensus of opinion with regard to the optimum temperature for the propagation of phage. In the bacteriophage laboratory of Dr. d'Herelle in Paris,<sup>5</sup> the phages are prepared at 33° C., the same temperature that is used by Dr. MacNeal of the Post Graduate Hospital.<sup>6</sup> Dr. Gratia at Liege prepares his phages at 37° C.<sup>7</sup> and Larkum of Lansing, Michigan, and Asheshov of India prefer the temperature of 22° C.<sup>8</sup> A study of this question in our laboratory has shown that a temperature of 32° C. to 33° C. was the most favorable for our staphylococcus phages. The experiments showed that phages prepared at 32° C. remained clear indefinitely, while bacteriophages made under identical conditions but at a temperature of 37° C. developed secondary growth after standing for one or two weeks at room temperature. No significant difference in potency as measured by plaque count and lytic action in broth was found between phages prepared at room temperature and at 32° C. However, room temperature preparation was abandoned after it was found that some strains of staphylococci, especially those just isolated from the body, grew at room temperature very slowly so that the control tubes were often found to be still clear after twenty-four hours. This might be a source of error. Besides, even a weak bacteriophage will often produce a complete visible lysis at room temperature. Thus, a weakening of phage can be more easily missed at room temperature than at 32° C. incubation.

#### D. BACTERIAL CULTURES

Now let us consider the cultures which should be employed for the propagation of bacteriophage. Some laboratories use a single strain of staphylococcus for the preparation of a number of staph-phages. Other laboratories employ a specific strain for each bacteriophage. These, after filtration may be mixed together. Still others propagate their phages against a mixture of bacterial strains after adding some freshly isolated strains to the laboratory stock strains each time that phage is made. Other workers, however, propagate their phages against separate individual strains and pool the filtrates.

Since we began the preparation of our phages we have tried several different methods. At first we followed the method of McNeal and Applebaum who prepared five phages at the expense of a single susceptible strain. After lysis had taken place the cultures were filtered and the filtrates pooled. This pooled phage produced complete visible lysis of the majority of strains at first but later, even though prepared in the same way, it lost its ability to lyse heterologous strains and then the homologous strains. We then shifted to another phage (BL) obtained from Gratia. When propagated on McNeal's stock strain it rapidly lost potency but when propagated on one of our stock cultures potency was maintained for over two months, and then it failed again. We then started with a new phage obtained from Larkum (B-59-A) and employed a stock culture for its propagation. For about three months this strain (1968) continued to undergo complete visible lysis and the plaque count showed the titer of phage to be  $10^{-8}$  or better. In the course of another month several freshly isolated cultures tested against this phage were found to undergo only partial lysis. We then went back to 12 laboratory strains previously susceptible and found that the phage was no longer able to dissolve completely all of the cultures. It was obvious that bacteriophage B-59-A, kept for a long time in contact with a single strain, had begun to lose its potency with respect to other strains. This was confirmed, for when the phage was tested against eight different cultures and the clear tubes were filtered and pooled, the new pooled phage was tested again against the same 12 strains of staphylococci. This time 11 cultures underwent complete visible lysis while one tube of broth was translucent. The increase in potency of phage B-59-A as the result of contact with several other strains of staphylococci was evident.

Since then we have repeatedly found that when a phage begins to lose its potency, it may be restored by propagating it against other suitable strains of staphylococci. This question will be studied in more detail and will be reported in a subsequent paper.

#### E. TESTING THE POTENCY OF BACTERIOPHAGE

The method most commonly used for testing the potency of bacteriophage against any given culture is to bring the bacteriophage into contact with a thin suspension of the bacteria in broth. One drop of a young culture of the bacteria is transplanted into two tubes containing 5 to 10 c.e. of plain broth of pH 7.4 to 7.8. This dilutes the culture down to a concentration of about 25 or 50 million organisms per cubic centimeter and gives a faint turbidity to the broth. Then to one of these tubes 0.1 c.e. to 1.0 c.e. of the phage filtrate is added. The other tube serves as a control. Both tubes are then incubated and the final results are read in eighteen or twenty-four hours in terms of the clarity of the suspension. If the fluid containing the phage is absolutely clear while the control shows good growth, the phage is considered to be potent.

This serves as a very rough gauge of phage activity and numerous methods have been devised to improve it. For example, potency has been measured by the rapidity of the clearing of the broth culture. Thus, in d'Herelle's laboratory of bacteriophage in Paris<sup>9</sup> phage is considered to be potent if one drop of it,

within three hours of incubation at 37° C., produces complete lysis of the bacterial suspension made with 5 drops of an eighteen- to twenty-four-hour-broth culture in 5 c.c. of plain broth.

In 1926 d'Herelle, himself,<sup>10</sup> preferred to determine the activity of bacteriophage by the actual count of "corpuseles." This estimate may be made by diluting the phage down through a series of decimal solutions in broth or saline and adding the bacterial suspension in the approximate concentration of 250 million per c.c. These tubes are well shaken and a measured quantity, approximately 0.02 c.c., is spread upon the surface of an agar slant or Petri dish. With the more potent phages there will be a complete inhibition of bacterial growth in the higher concentrations of phage. With the higher dilutions bacterial growth appears on the plate with clear areas scattered over the surface which represent foci of phage activity. These clear dots ("plaques") may then be counted and an estimate made of the number of phage "corpuseles" in the original suspension. According to Dr. Bulgakov of d'Herelle's laboratory in Paris, phage which yields a number of plaques when diluted  $10^{-6}$  is considered to be potent.<sup>11</sup>

Others measure the potency of bacteriophage by a method of successive dilutions in liquid medium proposed by Appelman.<sup>12</sup> This author considers that the higher the dilution of phage in which complete lysis of bacteria is obtained, the better the bacteriophage.

Still others combine Appelman's method with d'Herelle's. Transplantation of one drop of each dilution in broth is carried out on an agar slant. The potency is thus determined by the results of phage action both in liquid and in solid medium.

Sertie and Bulgakov,<sup>13</sup> on the basis of d'Herelle's idea that the potency of phage is proportional to the rapidity with which phage corpuseles multiply, offered the following method for testing the potency of phage: A culture grown for six hours at 36° C. on an agar slant, 18 mm. in diameter is washed off with 1.0 c.c. of broth; 0.05 c.c. of this suspension is then inoculated into 10 c.c. of broth and incubated for an hour at 36° C. At this time 1,000 corpuseles per c.c. of phage, estimated by preliminary plaque count, are inoculated into the broth and the culture is incubated further. At five-minute intervals during the first half hour a measured amount of the culture is plated and the plaques are counted. This is repeated at ten- or fifteen-minute intervals up to two hours, then at three, six, and twenty-four hours. The writers do not mention what the criterion of potency of phage is when measured by this method. Other methods of evaluating phage potency in general represent some modification or combination of the methods mentioned above.

The fact that many methods have been devised for determining the potency of bacteriophage indicates that none has been found to be entirely satisfactory. Furthermore, any method for determining potency in vitro must be correlated with clinical results if the laboratory is going to be of direct service in obtaining practical results in phage therapy.

Our difficulties in maintaining the potency of phage both in the laboratory and in the clinic induced us to study all of the various methods proposed for testing potency. In our earlier experience when one phage began to lose its



potency we were at a loss to know how to restore its potency and while attempting to find an adequate explanation we turned to another phage.

As we have stated above, when a phage began to yield poor results in the clinic it usually gave some indication of its loss of potency in the laboratory tests, for example, by failing to produce complete lysis first of heterologous strains and then of the homologous strain upon which it had been propagated. One phage *BL*, however, began to lose its potency clinically without any diminution

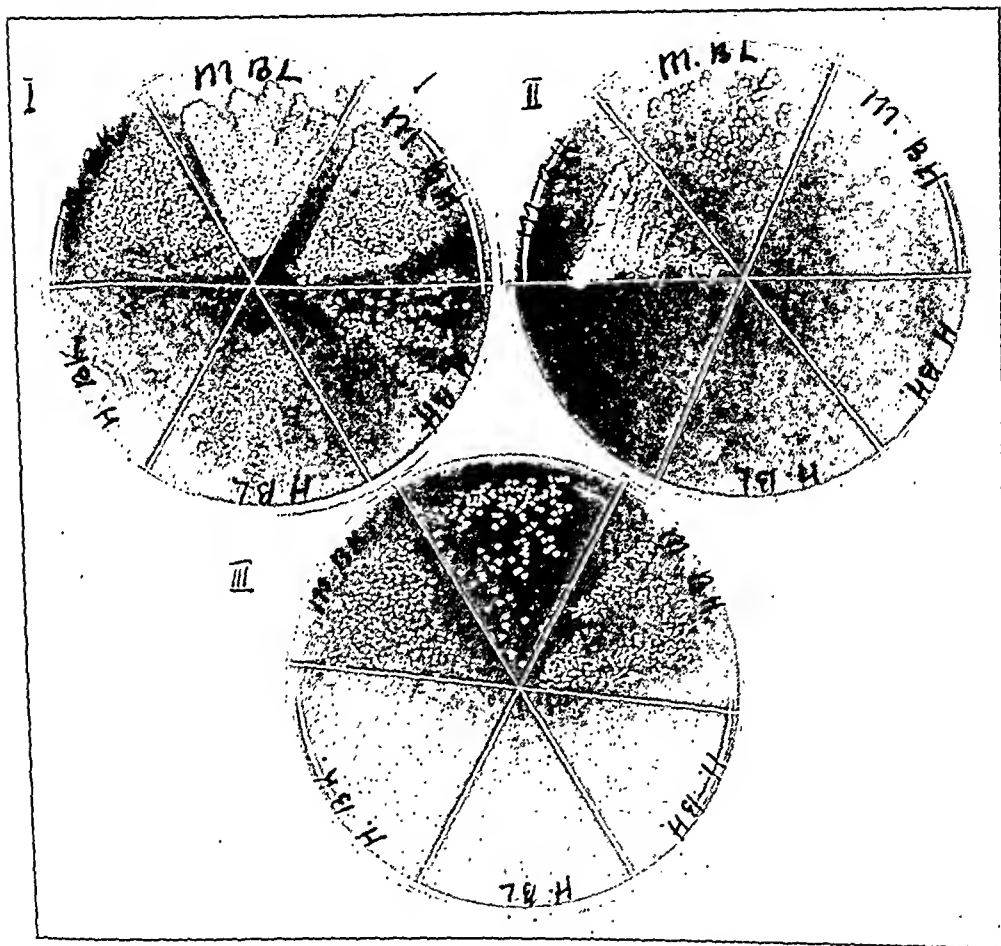


Fig. 1.—The character of bacterial growth from clear unfiltered phage suspension. The growth, obtained on the blood plate from the clear, unfiltered phage suspension: I, twenty-four hours; II, seventy-two hours; III, six days after its preparation. Staph-phages *BL*, *BH*, and *H* are prepared at the expense of strains *J* and *H* of staphylococci. With the strain *J*, all three phages gave an extensive growth of mostly normal and some degenerated-looking colonies from twenty-four-hour-old phage suspension and continued to do so after seventy-two hours and six days of standing at room temperature. The tubes from which the plating was done remained clear for about a month, at which time they were discarded. The same phages, prepared at the expense of the strain *H*, which is evidently much more susceptible, gave an abundant growth of degenerated-looking colonies from twenty-four-hour phage suspension, but the growth from seventy-two-hour suspension was considerably less extensive. In six days one of the phages did not give any growth at all and there was only one colony from one of the others and a few from the third.

of its lytic power in broth. But, when a loopful of the cleared culture, without previous filtration, was plated on blood agar we found to our surprise that the culture gave a profuse growth of individual nonconfluent colonies on the plate

(see Fig. 1). The colonies were yellowish and did not differ in any respect from normal staphylococcus colonies. When these colonies were smeared on slides and stained, the individual organisms appeared normal. When transplanted into broth and incubated, they gave a normal turbidity, and after being brought into contact with the same bacteriophage, they underwent complete visible lysis again. However, when this cleared culture was again plated on blood agar, it yielded typical staphylococcus colonies again. Some of the cleared tubes yielding growth were found to remain clear for a month while standing at room temperature, and during this period each time they were plated they gave an extensive growth of separated, normal looking colonies of staphylococci. When plain agar plates were inoculated instead of blood agar, growth was considerably less abundant and in some cases entirely absent (see Fig. 2). It was evident that blood agar was a better medium for the "invisible" staphylococci, or else the blood contained some element which inhibited the phage. However,

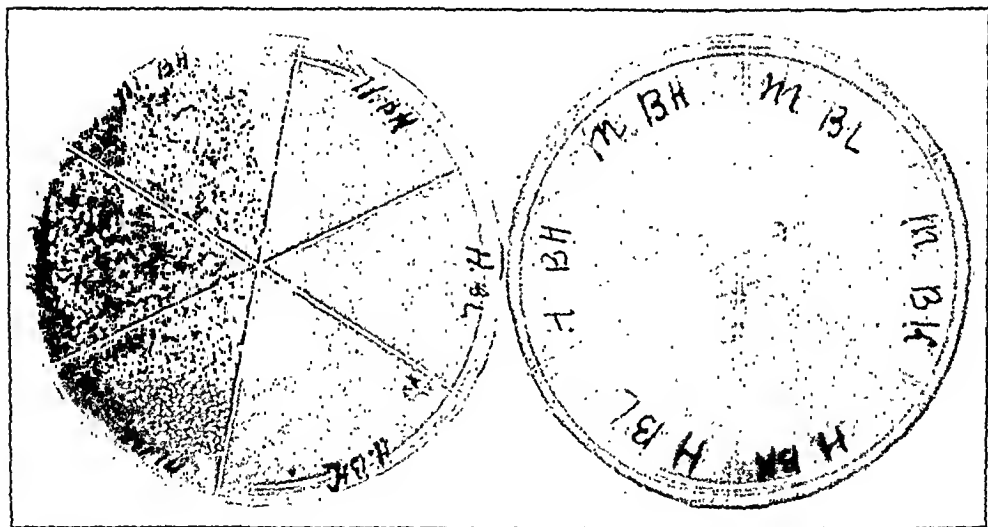


Fig. 2.—The difference of the degree of bacterial growth from phage suspensions on the plain agar and blood agar plates. Six-day bacteriophage suspensions of bacteriophages BH, BL, and H, prepared at the expense of staphylococci M and H are plated on the plain agar and blood agar plates. There is an abundance of mostly normal-looking colonies on the blood agar plate, while there is no growth on the plain agar plate.

the coincidence of this phenomenon with the clinical evidence of lack of potency suggested a reexamination of the clinically more potent phages by the same method.

A comparison of several different phages showed that some produced actual lysis of all of the bacteria and repeated plating failed to show growth while others cleared the cultures, but yielded growth of normal or abnormal colonies when the cleared tubes were plated. Still others failed to clear the bacterial suspensions at all. These three groups, we believe, represent three different degrees of potency, and yet they cannot be correlated exactly with other measures of phage potency. Bacteriophage may produce complete visible lysis of a large percentage of bacterial strains, and it may be of a high titer, as shown by titration through decimal dilutions in liquid medium or by any method of enumera-

tion of the bacteriophage corpuscles; still, if the phage is not really potent, cleared bacterial suspensions will yield growth of normal or abnormal colonies when plated on blood agar. This seems to be the first evidence of a fall in potency, for it is soon followed by a diminishing ability to produce complete visible lysis of bacteria in the test tube and by a drop in the number of bacteriophage corpuscles and a slowing of the rate of their multiplication. For this reason this simple method of testing phage potency is of practical significance and might be profitably employed whenever any clinical use is to be made of the phage. Although, when the phenomenon was first observed, as mentioned above, the colonies growing from the clear suspensions appeared normal, later experience with other phages made it evident that growth on blood agar from the unfiltered bacteria—bacteriophage mixtures vary considerably and each of the different kinds of colonies appearing on the blood plates has a different significance. The colonies may appear degenerated or they may be normal except for a change in color. The "moth-eaten" colonies indicate that the lysis of bacteria is slow. These colonies are partially transparent and contain particles of yellow pigment. They are usually hemolytic if the original culture is hemolytic. These colonies will be obtained from the most potent phages if the plating is done during the first or second hour of incubation after the bacteriophage is brought into contact with the bacteria. If the plating is done every ten to fifteen minutes after setting up the test, one will see these colonies gradually diminishing in number and under the action of a very potent phage they will disappear within two hours. With the less potent phages growth will appear up to twenty-four, forty-eight hours, and even later. Often the phages giving growth after twenty-four hours will not do so after forty-eight hours or later. Others will continue to yield some growth for several days, some in decreasing and others in increasing numbers depending on whether the phage or the bacteria dominate. In case of increasing bacterial growth, round normal looking colonies may appear without any sign of degeneration. These colonies may be yellow and look like the original culture except that they are usually nonhemolytic when the original culture is hemolytic. They may differ from an original culture also in color which may be grayish or greenish. If the colonies are greenish they are usually surrounded by a green zone of hemolysis. Only rarely do these colonies become confluent, although they may be very closely attached to each other. The green colonies are usually more dry than normal staphylococcus colonies, while the yellow or gray colonies have the consistency of normal staphylococcus colonies.

The development of one or another type of these abnormal colonies from a clear phage-bacteria suspension, after twenty-four hours of incubation usually indicates that the bacteriophage is losing its potency, or that the bacterial strain is acquiring resistance. The further use of this strain will result in a decrease in potency of the phage. In a large number of cases, especially if the appearance of these colonies has taken place for the first time, the tubes will remain clear for a long time, although when plated they will almost always give an abundant growth of bacteria. In some instances the number of colonies on the plate will gradually diminish over a period of days and finally disappear. The appearance of green colonies is followed by the de-

velopment of a visible cloudiness in the broth in a shorter period of time than when the cleared suspension yields the gray or yellow colonies.

#### F. THE DOSAGE OF BACTERIA FOR THE PROPAGATION OF BACTERIOPHAGE

In 1926 d'Herelle<sup>10</sup> recommended a heavy inoculum of bacteria approximating 250 million per c.c. of medium but a number of workers prefer a much smaller amount. MacNeal,<sup>14</sup> for example, in 1931 prepared his phages in bacterial suspensions estimated to contain 25 to 50 million per c.c. On one occasion when preparing one of the MacNeal phages, we inoculated six tubes containing 10 c.c. of broth with 25 million bacteria per c.c., while one tube by accident received 150 million to the c.c.; 0.5 c.c. of phage was then added to each tube (Table I). After twenty-four hours' incubation at

TABLE I

NUMBER OF STAPHYLOCOCCI PER CUBIC CENTIMETER	QUANTITY OF PHAGE INOCULATED	APPEARANCE OF THE MEDIUM AFTER 24 HOURS OF INCUBATION	GROWTH ON THE BLOOD AGAR PLATE INOCULATED WITH 1 LOOPFUL OF 24-HOUR PHAGE SUSPENSION	APPEARANCE OF THE BROTH AFTER 72 HOURS OF ROOM TEMPERATURE	APPEARANCE OF THE BROTH AFTER ONE MONTH OF ROOM TEMPERATURE
25 Million	0.5 c.c.	Clear	Abundant	Cloudy	Cloudy
25 Million	0.5 c.c.	Clear	Abundant	Cloudy	Cloudy
25 Million	0.5 c.c.	Clear	Abundant	Cloudy	Cloudy
25 Million	0.5 c.c.	Clear	Abundant	Cloudy	Cloudy
25 Million	0.5 c.c.	Clear	Abundant	Cloudy	Cloudy
25 Million	0.5 c.c.	Clear	Abundant	Cloudy	Cloudy
150 Million	0.5 c.c.	Translucent	None	Clear	Clear

32° C. all of the tubes inoculated with 25 million were clear, while the tube containing 150 million c.c. bacteria was translucent. When a drop from each tube was plated on the blood plate, however, there was an abundant growth from the clear tubes, while the tube inoculated with 150 million per c.c. did not yield a single colony. All of these tubes were left on the desk at room temperature, and after standing seventy-two hours, the clear tubes became slightly cloudy, while the tube inoculated with the larger amount of bacteria became clear and remained so for a month. In an effort to bring light upon this problem, we prepared bacterial suspensions with varying amounts of organisms, estimation of the number being made by the "turbidimeter" method of Gates.<sup>15</sup> The experiment was set up with a series of tubes inoculated with 25 million, 150 million, and 300 million per c.c. and the result confirmed the accidental finding (Table II). The tubes containing the smaller inoculum be-

TABLE II

NUMBER OF STAPHYLOCOCCI PER CUBIC CENTIMETER	QUANTITY OF P. PHAGE INOCULATED	APPEARANCE OF BROTH AFTER 24 HOURS	GROWTH ON THE BLOOD AGAR INOCULATED WITH 24-HOUR PHAGE SUSPENSION	TITER OF PHAGES AS ESTIMATED BY PLAQUE COUNT
25 Million	0.5 c.c.	Clear	Abundant	10-7
150 Million	0.5 c.c.	Translucent	None	10-9
300 Million	0.5 c.c.	Translucent	None	10-8

came clear but yielded growth on the plate while the tubes with 150 million c.e. and 300 million c.e. remained translucent for twenty-four hours but yielded no growth and later the tubes cleared. When the potency of bacteriophage prepared at the expense of these three amounts of staphylococcus was tested by the count of plaques on agar, the phage prepared at the expense of 150 million c.e. was found to possess a higher titer than either of the other quantities of inoculum. These experiments were then repeated with another phage B-59-A obtained from Dr. Larkum and another strain of staphylococcus. A series of 10 tubes containing 10 c.e. of plain broth was used (Table III). The amount of

TABLE III

NUMBER OF STAPHYLOCOCCI PER CUBIC CENTIMETER	QUANTITY OF B-59-A PHAGE INOCULATED	APPEARANCE OF BROTH AFTER 24 HOURS	GROWTH ON THE BLOOD AGAR	APPEARANCE OF BROTH			TITER OF PHAGE AS TESTED BY PLAQUE COUNT
				72 HR.	96 HR.	2 WK.	
25 Million	0.1 c.e. = 10 million corpuscles	Clear	Abundant	Slightly cloudy	Cloudy	Cloudy	$10^{-6}$
75 Million	0.1 c.e. = 10 million corpuscles	Clear	Moderate	Clear	Slightly cloudy	Cloudy	Not tested
150 Million	0.1 c.e. = 10 million corpuscles	Clear	Absent	Clear	Clear	Clear	$10^{-8}$
225 Million	0.1 c.e. = 10 million corpuscles	Translucent	Absent	Clear	Clear	Clear	Not tested
300 Million	0.1 c.e. = 10 million corpuscles	Translucent	Absent	Clear	Clear	Clear	$10^{-7}$

inoculation ranged as follows: 25, 75, 150, 225, and 300 million organisms per c.e. two tubes being used for each dose of organisms; 0.1 c.e. of phage containing approximately 10 million "corpuscles" was added. The tubes were then incubated at 32° C. for twenty-four hours. When examined, it was found that the tubes inoculated with 25, 75, and 150 million bacteria per c.e. were clear, while those prepared at the expense of 225 M/c.e., and 300 M/c.e. were translucent. A drop from each tube was then plated on blood agar plates and incubated overnight. Meanwhile one set of the tubes was filtered and another set left at room temperature for further observation. When the plates were examined on the following day the parts of the plate inoculated with one drop from the tubes receiving the smallest dose of organisms yielded a very rich growth of separate colonies. The colonies were considerably less abundant from the tube inoculated with 75 M/c.e. Not a single colony grew out of the tubes inoculated with 150, 225 M/c.e. and 300 M/c.e. of bacteria. The series of tubes which were left at room temperature remained clear for forty-eight hours. Then slight cloudiness was noticed in the tube inoculated with 25 M/c.e. bacteria and a day later in the 75 M/c.e. tube. The other tubes, two of which remained slightly translucent, did not show any cloudiness and did not grow on the plate for a period of two weeks after which they were discarded.

The phages prepared at the expense of 25 M/c.e., 150 M/c.e., and 300 M/c.e. were tested for their potency by the plaque count. Plaques appeared in the eighth decimal dilution of the phage prepared at the expense of 150 M/c.e. while the limit of the phage prepared with the 300 M/c.e. inoculum was the seventh dilution and the limit of the phage prepared with the 25 M/c.e. inoculum

was the sixth dilution. These experiments were repeated a number of times (one of them is presented in Fig. 3) and according to the plaque count method of testing potency, the optimum inoculum was found to be from 150 to 250 million organisms per c.c. If more than 250 M/c.c. of bacteria were used it required longer than twenty-four hours' incubation at 32° C. to produce lysis and complete clearing seldom occurred. Usually there was slight translucence. However, the plaque count of phage produced with these larger quantities of inoculum was usually only slightly lower than that produced by the phage

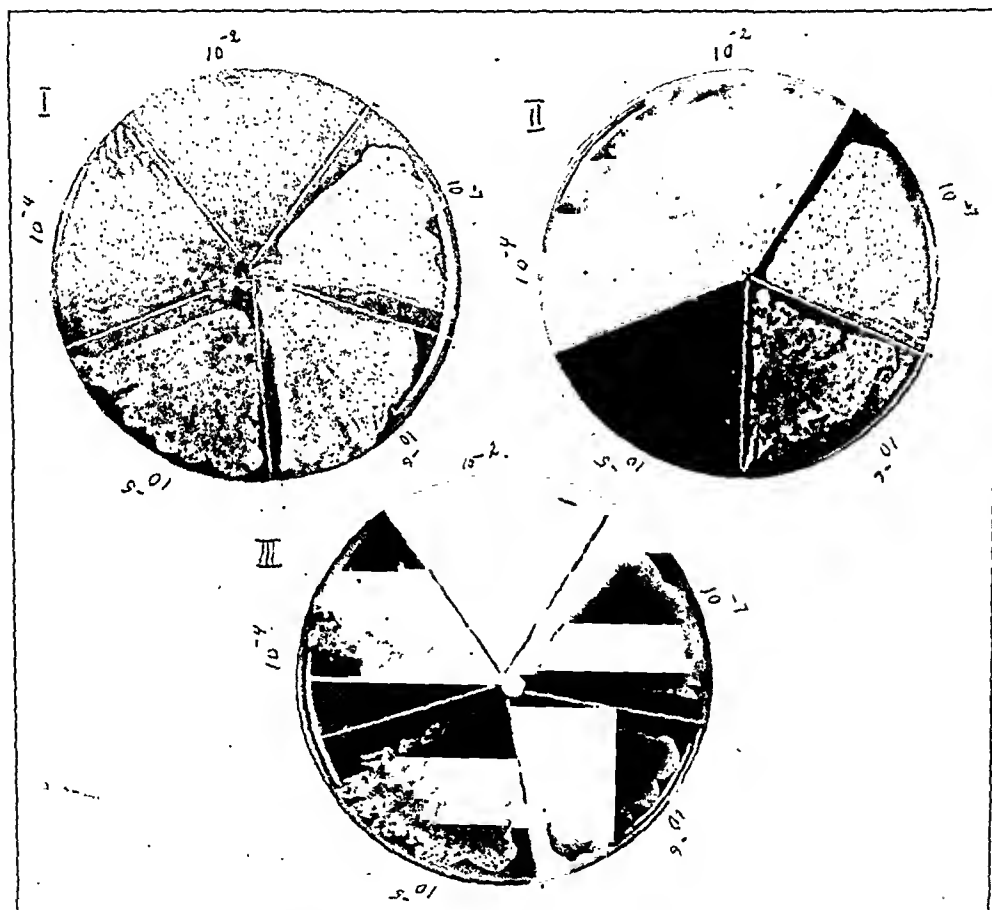


Fig. 3.—Potency of bacteriophage prepared at the expense of 25 million, 150 million and 300 million per c.c. as estimated by plaque count. Each plate is divided in five parts which are inoculated with  $10^{-2}$ ,  $10^{-4}$ ,  $10^{-5}$ ,  $10^{-6}$ ,  $10^{-7}$  dilutions of phage. One can see that the phage prepared at the expense of 150 million per c.c. contains the largest number of corpuscles. This is followed by phage prepared at the expense of 300 million of staphylococci per c.c. and the phage propagated at the expense of 25 million per c.c. contains the smallest number of corpuscles.

propagated at the expense of 150 to 250 million organisms per c.c. When the inoculum was above 500 M/c.c. several days were usually required to produce lysis and the potency of the phage was usually lower. On the other hand, phages prepared at the expense of a smaller amount than 100 M/c.c. were always less potent and the potency decreased in proportion to the decrease in the

inoculum. Thus the titer of phage prepared at the expense of 1,000 bacteria per c.e., never exceeded  $10^{-5}$  which meant that there had been no propagation of the phage originally added to the suspension.

#### G. THE QUANTITY OF BACTERIOPHAGE INOCULATION FOR THE PROPAGATION OF PHAGE

Concerning the quantity of bacteriophage which should be used for phage propagation d'Herelle<sup>10</sup> states that "the quantity of bacteriophage is a negligible factor." Employing dilutions beginning from  $10^{-1}$  up to  $10^{-11}$  of dysentery phage against 100 M/c.e. bacteria d'Herelle invariably obtained 4+ lysis. There was sometimes a delay in the development of lysis with the highest dilutions but in the lower dilutions lysis within eighteen to twenty-four hours was always complete. D'Herelle came to the same conclusion with respect to other phages including staphylococcus phage. However, in 1922, Ellis<sup>15</sup> described somewhat different observations. This author found that a different degree of lysis was obtained when different amounts of bacteriophage were used. There was complete lysis of the bacterial suspension with a small quantity of phage, while only partial lysis was obtained when a larger inoculum of phage was employed. Somewhat similar observations were made by Gohs.<sup>16</sup> This writer, testing a bacteriophage, preserved for a year, found that when the quantity of a bacteriophage varied between 1/50,000 and 1/10,000,000 of a drop, lysis was complete, but when the quantity of the phage employed was from 5 drops to 1/10,000 of a drop the bacteria remained alive.

Carrying out numerous titration tests with staphylococcus and other bacteriophages in our laboratory we have found that complete lysis may be obtained in dilutions of  $10^{-10}$  or even higher, while the dilution  $10^{-1}$  (and occasionally the dilution  $10^{-2}$ ) may fail to clear the bacterial suspension. This cloudiness was at first considered to be due to a contamination, but the constancy of this phenomenon led us to prove that such was not the case. Four tubes of broth (Table IV) were inoculated with 25 million bacteria per c.e.,

TABLE IV

NUMBER OF STAPHYLOCOCCI PER CUBIC CENTIMETER	QUANTITY OF PHAGE INOCULATED	APPEARANCE OF BROTH AFTER 24 HOURS	GROWTH ON THE BLOOD AGAR PLATE	APPEARANCE OF THE TUBES AFTER 92 HOURS
25 Million	0.1 c.e.	Cloudy	Abundant	Cloudy
25 Million	0.01 c.e.	Clear	Abundant	Cloudy
25 Million	0.001 c.e.	Clear	None	Clear
25 Million	0	Very cloudy	Very extensive	Cloudy

and phage was added in amounts ranging from 0.1 c.e. to 0.001 c.e. in three tubes, containing respectively approximately ten million, one million, and one hundred thousand "corpuscles." The fourth tube was used for control. After twenty-four hours of incubation the tubes inoculated with the  $10^{-2}$  and  $10^{-3}$  c.e. dilutions of phage were clear, while the one inoculated with the  $10^{-1}$  c.e. dilution displayed only partial lysis. When a drop from these tubes was plated on agar there was confluent growth from the tubes inoculated with the  $10^{-1}$  and  $10^{-2}$  dilutions of phage and no growth from the tube inoculated with the  $10^{-3}$  dilu-

tion. When examined after seventy-two hours at room temperature the tubes inoculated with  $10^{-1}$  and  $10^{-2}$  dilutions of phage were found cloudy, while one with  $10^{-3}$  dilution of phage was clear.

Another series of five tubes (Table V) was seeded with 250 million bacteria per e.c. and one of them was inoculated with 1.0 e.c., another with 0.5 e.c., the

TABLE V

NUMBER OF STAPHYLOCOCCI PER CUBIC CENTIMETER	QUANTITY OF PHAGE INOCULATED	APPEARANCE OF BROTH AFTER 24 HOURS	GROWTH ON THE BLOOD AGAR PLATE	APPEARANCE OF THE TUBES AFTER 72 HOURS
250 Million	1.0 e.c.	Clear	None	Clear
250 Million	0.5 e.c.	Clear	None	Clear
250 Million	0.1 e.c.	Clear	None	Clear
250 Million	0.01 e.c.	Slightly cloudy	None	Clear
250 Million	0.001 e.c.	Cloudy	None	Clear

third with 0.1 e.c., the fourth with 0.01 e.c., and the fifth with 0.001 e.c. of the same phage. There was complete lysis in the tubes inoculated with 1.0 e.c., 0.5 e.c., and 0.1 e.c. of phage, while lysis in the tube inoculated with 0.01 e.c. was only partial (3+) and in the one inoculated with 0.001 e.c. of phage lysis was practically absent. However, when all five tubes were plated on blood agar none yielded any growth. When examined later the tubes inoculated with 250 million bacteria per e.c. and 0.01 and 0.001 e.c. of phage were found to be completely clear.

This experiment clearly demonstrated that the quantity of bacteriophage is an important factor in the propagation but it is particularly important not to use large amounts of bacteriophage such as 0.5 e.c. or 0.1 e.c. per 10 e.c. of medium when a small inoculum of bacteria (25 M/e.c. or below) is used. In cases when the number of bacteria ranges between 100 and 250 million per e.c. less care as to the quantity of bacteriophage is required. In this case amounts of 1 e.c., 0.5 e.c., 0.1 e.c., and 0.01 e.c. will produce complete lysis of bacteria equally well. However, if higher dilutions of bacteriophage are employed there may be some delay in completing lysis.

This relationship of the quantity of bacteriophage to the number of organisms has been observed with several staphylococcus bacteriophages obtained from different sources and the proportions necessary for the development of the optimum potency varies with different strains of bacteria and different races of phage but in general the best results will be obtained with 150 to 250 million organisms per e.c. and 0.1 or 0.01 e.c. of phage in 10 e.c. of broth.

#### SUMMARY AND CONCLUSIONS

The problem of maintaining and testing the potency of staphylococcus bacteriophage has been studied from the point of view of the media to be employed, the temperature of incubation, the strains of bacteria to be used, and finally the proportion of the number of bacteria to the quantity of bacteriophage. We have also considered the various criteria of potency. Our experiments seem to indicate the following:



1. "Savita" medium is superior to plain meat infusion broth chiefly because of the relative infrequency of uncomfortable reactions in the patient, but laboratory tests indicate that the broth phages are more potent.

2. The optimum temperature for the propagation of *staphylococcus bacteriophage* seems to be 32° or 33° C.

3. Although the potency of phage may be maintained for several months by its propagation at the expense of a single strain of bacteria, it may sooner or later lose its potency thereby, and have to be restored by propagation against another suitable strain.

4. More potent phages are obtained by using large inoculations of bacteria (150 to 250 million per c.c.) and small inoculations of phage 0.1 to 0.01 c.c. (approximately 1 to 10 million corpuseles) in 10 c.c. of medium, than with smaller quantities of bacteria and larger amounts of phage.

5. The earliest indication of loss of potency is the appearance of growth on blood agar from the cleared bacterial suspension. This is followed by a failure to produce lysis and a slowing of the rate of multiplication of phage corpuseles.

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## MALARIA STUDIES IN GREECE\*

### A MODIFICATION OF THE UHLENHUTH-WEIDANZ PRECIPITIN TEST FOR DETERMINING THE SOURCE OF BLOOD MEALS IN MOSQUITOES AND OTHER INSECTS

J. B. RICE, M.D., AND M. A. BARBER, PH.D., CAVALLA, GREECE

THE aim of the modification of the Uhlenhuth-Weidanz precipitin test, here described, is to secure economy of time and materials. One cubic centimeter of each animal precipitin serum suffices for the test of 700 or 800 mosquito blood meals with a gain in accuracy as compared with the older methods. One person with an assistant to clean apparatus can do more than 1,000 tests in one day. In investigations dealing with the source of mosquito blood meals, the number of variables is great, and abundant data are especially desirable. Anopheles are usually plentiful; the main obstacles in the past have been the expense of the precipitin sera and the time required to do the tests.

#### COLLECTION OF MOSQUITO BLOOD MEALS FOR TESTING

The fresher the blood to be tested, the better. In winter, mosquitoes kept alive nearly four days at low room temperature may yield blood which gives a fair test. In warm weather, blood often becomes black, scanty, or otherwise unfit for the test in less than one day. Our summer routine, especially useful where the mosquito collecting place is at some distance from the laboratory, is as follows: Collections are made as early in the forenoon as possible. Collections from each bedroom, stable, or other locality-unit are transferred from a narrow catching tube to tube vials, each about 2 cm. wide and 5 cm. high. These are tightly corked and put into a large thermos jug, porcelain lined and containing an abundance of ice. Vials are numbered and each number is entered on a card describing the locality in which the mosquitoes were taken. Vials of greater size may be used for larger collections, but many mosquitoes can be put into one vial of the size described.

It is best to remove the blood from the mosquito stomachs on the day of collection, but fresh blood will keep in a well-iced thermos jug for twenty-four hours. We often use the same mosquitoes for the precipitin test and for the dissection of salivary glands for sporozoites, so it is convenient to keep the specimens until both tests can be made. The blood may, of course, be removed immediately after collection or the mosquito may be dried, blood and all. The extraction of the serum from a dried insect, however, is less satisfactory than from blood removed when fresh, especially where large numbers of tests are being done.

In the laboratory, the mosquitoes are lightly chloroformed and the blood from each stomach is expressed on filter paper, preferably of a harder sort.

\*Received for publication, July 27, 1934.

It is best not to press the blood too deeply into the filter paper, as soaked-in blood does not yield its serum so well. We prefer circular filters, such as Whatman No. 5, 9 cm. in diameter. The "blood-spots" are arranged at the margin, and labels are written at the center of the sheet. The sheets are kept in tightly closed tin boxes. It is possible to keep dried blood specimens many months, at all events when they are stored in a cold, dry place.

The blood specimens are kept until many hundreds accumulate. Then on the day of testing, the spots are cut out and one is placed in each of the compartments of a long tray (Fig. 1). The tray may be made of tin or other metal, but must be so carefully japanned or otherwise coated that no metal is exposed. Each compartment is 5.5 cm. long, 3 cm. wide at the top, and 2 mm. wide at the bottom; the cross-section is thus nearly an isosceles triangle.

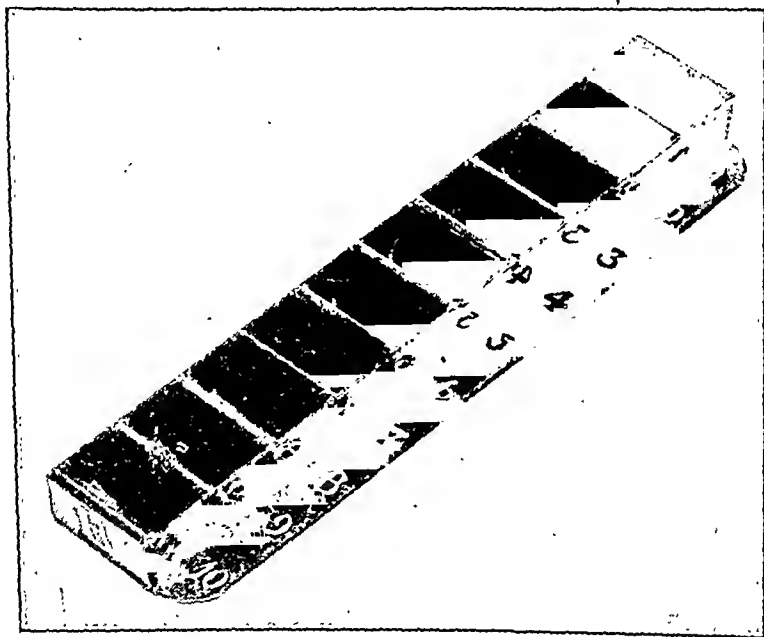


Fig. 1.—Tray with compartments for extracting serum from mosquito blood meals.

Three cubic centimeters of physiologic salt solution are then added to each specimen. More or less salt solution may be added depending on the amount and freshness of the blood, in respect to which blood meals vary widely. Our aim in this test is merely to determine the chief source of the blood meals of a considerable group; good specimens may be selected and all doubtful reactions thrown out. Using a precipitin serum prepared as described below, group reaction due to antigens common to different species of animals cause few or no cross reactions. Under these conditions, the permissible degree of dilution of the antigen is a wide one, and 3 c.c. of salt solution answers for practically all tests of mosquito blood meals.

The blood spots are allowed to soak for one hour at room temperature, the trays being shaken from time to time. It is not necessary to filter this solution, but it should be allowed to settle a short time before use so as to give a clear supernatant fluid in each compartment.

## PREPARATION OF THE PRECIPITIN SERUMS

We obtain serums furnished by the Instituto Sieroterapico of Milan, Italy. These serums are supplied in either a liquid or a dried state. The dried serums used by us were serviceable, but they contained a fine precipitate which had to be removed by filtration before use. It is possible that this precipitate was caused by bleeding the animals too soon after feeding. The liquid serums are preferable, but should be ordered long beforehand. They are furnished sealed in ampules and may be preserved many months at refrigerator temperature. We use antisera for man, pig, horse, sheep, and cow. The number and species of animals depend, of course, on the nature of the experiment and the region where the tests are made.

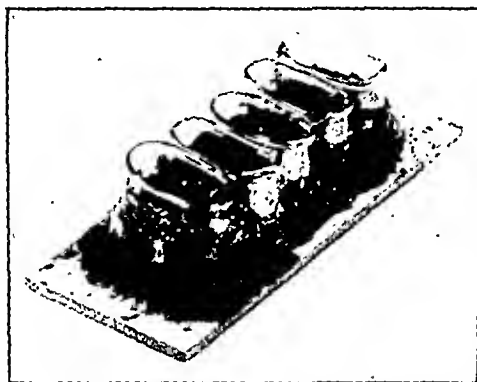


Fig. 2.—Group of glass dishes for the diluted precipitin serums.

In preparation for the test, the precipitin serums are diluted as follows: To each part by volume of serums are added seven parts of the following diluent:

Sodium chloride	4.25 gm.
Glycerine	166.0 c.c.
Phenol	2.5 c.c.
Distilled water	330.0 c.c.

This diluent should be perfectly clear; if it is not, it must be filtered before it is added to the serums. The glycerine should be nearly neutral. Its purpose is to add to the specific gravity of the fluid. The phenol, added to prevent the growth of yeasts or moulds, may be omitted if the diluent is used at once. With the phenol ingredient, the diluent will keep indefinitely; and after the addition of the precipitin serums, the mixture will be effective for as long as ten months without appreciable loss of strength when kept at ice box temperature and protected from access of air by overlaying with paraffin oil. One test sample kept well for weeks at summer room temperature.

The titers of the various batches of serums are marked on the ampules "1:1,000, 1:2,000, and 1:3,000." However, several samples tested in this laboratory have shown the titer to be closer to 1:5,000, and no variation in the amount of dilution has had to be made for different batches of serum. It is probable that if serums are used which have a lower titer than that mentioned above, the dilution will have to be correspondingly decreased; it will have

to be increased if the serums have a higher titer. In any case, a few simple tests on known blood meals will serve to indicate the proper dilution for any given precipitin serum.

Immediately before the test, the diluted precipitin serums are placed in a "battery" of small glass dishes cemented on an ordinary glass slide (Fig. 2). Each dish receives a different animal precipitin serum, and these are always arranged in the same order, that is, man, pig, horse, sheep, and cow.

The diluted stomach bloods of the mosquitoes and the precipitin serums are brought into contact by means of capillarity in glass tubes. These tubes, each about 6.5 cm. long with a 2 mm. inside diameter, are sealed between two glass slides, one tube for each animal for whom the test is being made. Thus

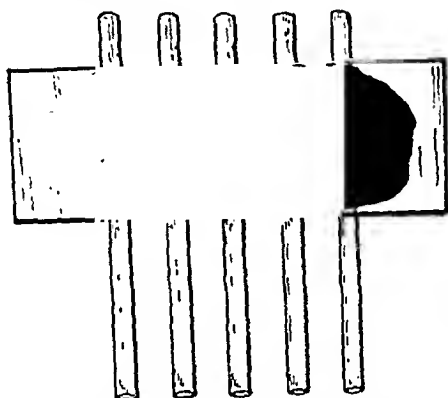


Fig. 3.—"Card" of capillary tubes in which antigen and precipitin serums are brought into contact.

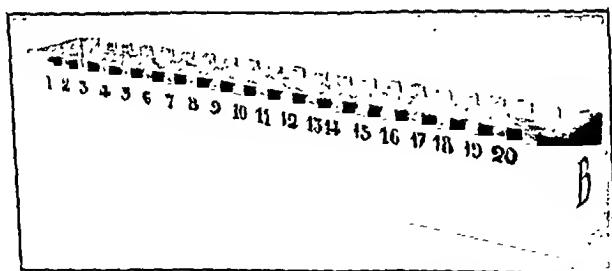


Fig. 4.—Rack for holding "cards" of capillary tubes.

there is a 5-tube unit, which we call a "card" for want of a better term (Fig. 3). The tubes and the small dishes (Fig. 2) are so spaced that each tube of a card will touch approximately the center of the precipitin serum in a dish when the tubes are lowered simultaneously. Only the long ends of the tubes are used, so that the material employed to seal them between the slides need not be transparent; ordinary black asphalt cement will do.

In performing the test, the card is first brought into contact with the antigen (the diluted stomach blood) in one compartment of the tray (Fig. 6, a), all tubes receiving the same antigen simultaneously. The liquid enters by capillarity and rises a distance of about 1 cm. Then all tubes are touched to a layer of wet absorbent cotton (Fig. 6, b) to draw out one-third of the

antigen and to wipe the bottoms of the tubes. Then the tubes are touched to the diluted precipitin serums, all tubes simultaneously. The precipitin serums flow into the tubes until the total height of the column, composed of antigen plus precipitin serum is again about 1 c.c. Whether touching antigen, absorbent cotton, or precipitin serums, a contact of a fraction of a second suffices.

There is now a layer of each animal precipitin serums under a layer of antigen composed of the diluted blood of one mosquito stomach. The reaction, consisting of a ring at the zone of contact of the two liquids, often appears within a few seconds, but it is best to read the result after about 20 minutes. It is convenient to do about 80 stomachs, then read the results in all. Cards are held in racks (Fig. 6, *c*) while awaiting the reaction and reading.

In preparing the absorbent cotton (Fig. 6, *b*) a flat layer is wet with physiologic salt solution, and placed between glass plates; then, while the cotton and one plate are held together, the other plate is slipped aside, a process known to photographers as "squeegeeing." The cotton layer lying on one glass plate is now placed in a shallow glass dish and physiologic salt solution is added until its level comes just below the level of the top of the cotton. The height of the cotton layer above the salt solution will regulate the amount of antigen drawn out, and the level of the salt solution may be changed if too much or too little antigen is withdrawn. It is essential that the cotton be nearly neutral, as the presence of acid in any considerable amounts in any part of the apparatus is likely to cause a nonspecific precipitate due to the coagulation of the precipitin serum.

It would seem that there would be danger of false reactions through mixing antigens from different mosquitoes in the cotton layer. Repeated tests for this source of error indicate that it does not exist. One may, of course, touch a different part of the cotton layer for each mosquito, but this precaution does not seem to be necessary.

The diluted precipitin serums in the row of glass dishes is dipped until all is used. We have made careful tests to determine if a sufficient amount of antigen, coming from the tubes through repeated dippings, is left in the precipitin serums to impair the test. We found no evidence of such source of error. The inrush of the precipitin serums apparently carries with it any antigen which may have escaped the wiping on the absorbent cotton. In any case, a contamination with antigen would result in a general cloudiness of the precipitin serum, a condition readily detected and not easily confused with a positive reaction.

A strong reaction may be read by simply holding the card before a bright light. But we have found it advisable to use a sort of observation box (Fig. 6, *d*). This box is of wood, and is about 32 cm. long, 9 cm. wide, and 6.5 cm. deep. It is painted black and is closed except for one end, which is left open for the eyes of the observer, and a space at the top of the other end about 8 cm. long. At this end there is a notch for holding the card. It is convenient, and, where one uses a one-eighth dilution of the precipitin serum, necessary, to include in the box an ordinary square reading glass to magnify the ring.

The card is suspended in the open end of the box and held almost under a strong electric light for reading. This arrangement avoids confusion due to reflected light.

The rings formed where precipitin serums and antigen meet are narrow but sharp. The group reaction, cow + sheep, rarely if ever occurs when one uses the diluted precipitin serums as described here. An attempt is made to have the tubes perfectly clean (see method of washing described below), but if there is ever suspicion of confusing a ring with some precipitate on the outside of the tube, the card may be tipped slightly and the angle of the ring will change, that of the artifact remaining constant.

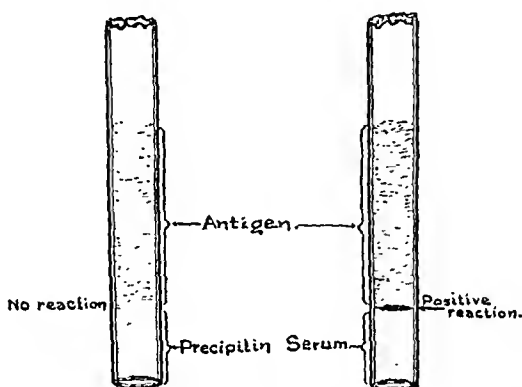


Fig. 5.—The lens-shaped ring appearing in a positive reaction.

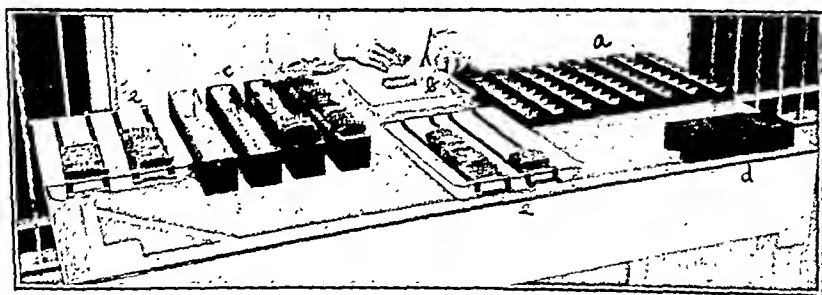


Fig. 6.—The various kinds of apparatus arranged for making a large number of precipitin tests. *a*, Trays for serum from mosquito blood meals. *b*, Glass dish containing wet absorbent cotton. *c*, Racks for holding "cards." *d*, Observation box. *e*, Large dishes in which "cards" are kept when not in use.

Of great use in recognizing the precipitin ring is its biconvex lenslike form (Fig. 5). The reason for this form is seen when a test is made with the diluted precipitin serum colored with eosin or other aniline dye. The top of the precipitin serum column is at first conical, not flat-topped. Owing to its greater specific gravity the cone soon flattens down, leaving a lens-shaped ring.

The washing of the tubes in the card is important if proper capillarity is to be obtained in tubes of so large a caliber. They are washed thoroughly in sulphuric bichromate solution and are left in the solution overnight. All traces of this cleaning mixture are then washed out with tap water. Afterward, the cards are suspended in a large dish of distilled water where they

are left until they are needed. When they are to be used the distilled water is shaken out with a sharp jerk and the card is immediately brought into contact with the antigen without allowing the tubes to become dry. Immediately after the test is read, the contents of the tubes are shaken out and the cards are placed in a second dish containing distilled water or tap water. If tap water is used, about 3 drops of concentrated hydrochloric acid per liter should be added. Just enough acid is added to prevent the carbonates of the tap water from forming a precipitate on the outside of the tubes. Many tap waters will need less acid or none at all. Cards are left in this water until ready for the next test. One can use cards in this way all day. At night, or at the close of a series of tests, they are put back into the sulphuric acid-bichromate solution. On two occasions a precipitate has formed on the tubes which could not be removed by the acid bichromate solution. This precipitate was readily removed, however, by suspending the tubes in a strong sodium hydroxide solution for a few minutes.

Glass dishes (made by any glass manufacturer) 26 by 7 by 7 cm. are convenient for keeping tubes in the cleaning solution. For the distilled water or tap water, large dishes of any kind will do if they are provided with racks suspended from the margin for holding the cards (Fig. 6, *e*).

#### PROOFS OF THE RELIABILITY OF THIS MODIFICATION

In working out this modification, we devised a microscopic method for comparison. On large slides (those 2.5 by 8 cm. are good) small drops of undiluted precipitin serums are placed, several rows to a slide, and each row consisting of a different animal antiserum. Wax pencil lines separate the droplets. The droplets are allowed to dry and may then be used at once or stored in the refrigerator, protected from access of air, where their properties will keep for several weeks. In making the test, a drop of diluted antigen from one mosquito is placed on each dried droplet in a transverse row, that is, one comprising serums of all the animals for which the test is being made. On a second row, another antigen is placed, and so on. The slide is then put in a moist chamber for half an hour and the result finally read under the low power of a compound microscope. A positive result, consisting of an opaque cloud of precipitin, is precise and clear, especially if antigen and precipitin serums are free from dirt.

We have compared many series of tests by both the microscopic and the macroscopic capillarity modifications. The results agree remarkably well; perfectly, when proper mosquito bloods are available.

We have also tested the reliability of both modifications by the use of known serums of various animals and at various dilutions. But best of all have been tests on mosquitoes which have fed on animals, the species of which were known. In more than 1,100 experiments, mosquitoes were exposed to known pairs, man and cow or man and donkey, and the stomach bloods subsequently tested by the macroscopic capillarity method. In no case did a



reaction appear for any animal other than for one of those bitten. Of the two methods, the macroscopic capillarity method is preferred, since it is just as precise and far more convenient.\*

Doubtful reactions are thrown out, or, if material is valuable, the test is repeated. Usually one can get freshly fed mosquitoes for an abundance of unequivocal tests. The purpose of such tests to determine animal preferences of insects does not usually require the identification of blood meals that are scanty or otherwise inadequate.

All of our apparatus is homemade or bought in local markets, except the battery of dishes for the precipitin serums and some of the plain glass dishes for washing the cards. These were made to order by glass manufacturers in Rome or Athens. If this method proves as serviceable to others as it has to us (we have done over 20,000 tests), it may be that some dealer will eventually furnish complete sets of apparatus at moderate cost.

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## NOTE ON THE FIXATION OF SMEARS FOR BACTERIOLOGIC STUDY\*

FLORENCE L. EVANS, PH.D., NEW ORLEANS, LA.

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IT HAS been found by experience that the ordinary method of heat fixation is inadequate in the preparation of smears from certain types of material. Cultures of anaerobic spore formers are especially difficult to deal with, as the material dries in a varnish-like layer which washes off during the most careful staining procedures. Special methods of fixing such smears that have been advocated have been tried, but the results have not been altogether satisfactory.

The following method of heat fixation has been found uniformly successful in the preparation of smears from the anaerobic microorganisms and has been used exclusively for the past two years. The method is extremely simple and requires no equipment beyond that customary in any laboratory.

The film is prepared by smearing the material on the slide in the customary manner. The slide is placed in the incubator at 37° C. for one-half to three-quarters of an hour. It is then removed and put in the hot air oven at a temperature of 170° C. for ten minutes. Films prepared in this way can be stained in the usual manner and remain firmly fixed to the slide.

Slides of pathologic material containing organisms that are apt to multiply at 37° C. can be fixed in the 55° C. incubator for fifteen minutes and then in the hot air oven. This method has been found very useful for material containing blood serum or tissue, but it is not as satisfactory for anaerobes as the first method described.

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\*From the Department of Pathology and Bacteriology of the Medical Center of Louisiana State University and the Charity Hospital of Louisiana.  
Received for publication, August 1, 1934.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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**BLOOD**, Study of, in Normal Pregnancy, Dieckmann, W. J., and Wegner, C. R. Arch. Int. Med. 53: 529, 1934.

In individual subjects, both plasma and whole blood cholesterol remain fairly constant, although within rather wide limits, and they are not affected by food unless prolonged alterations in diet are maintained.

Determinations of plasma cholesterol and plasma volume were made on various groups of women and also on the same women for the different periods of pregnancy. The following conclusions are warranted:

1. The variations and range of cholesterol in the same woman during pregnancy may be tremendous. The majority show an increase, which is apparent at from ten to fifteen weeks' gestation.

2. There is an average increase of 23 per cent at term and a decrease of 27 per cent at eight weeks postpartum.

3. Cholesterol calculated as grams per kilogram shows an increase of 33 per cent at term, and a decrease of 39 per cent at eight weeks postpartum.

Variations in the total amount of cholesterol indicate an increase of 33.9 per cent at from twenty-six to thirty-five weeks of gestation, and an increase of 27.9 per cent at term. After delivery there is a constant decrease, amounting to 21.2 per cent at eight weeks postpartum.

The increase in both the concentration and the total amount of cholesterol is probably not preparatory for lactation but is associated with other changes in the chemical equilibrium, which is so markedly disturbed in pregnancy. Further work is necessary to determine whether the increase in cholesterol in pregnancy is caused directly by the pregnancy or is the result of the other changes in the blood.

**BLOOD SEDIMENTATION**, A Simplified Technique for, Van Antwerp, L. D. Am. Rev. Tuberc. 29: 214, 1934.

The method is as follows: 0.2 c.c. of a sterile 3 per cent solution of sodium citrate is drawn from a serum bottle under sterile technic. The needle is then removed from the serum bottle cap and, following a venous puncture from a suitable vein, the syringe is filled to the 2 c.c. mark with blood, and removed from the vein. A bubble of air is then expelled, the needle removed and replaced by a cap, and the capped syringe placed in a test tube rack for observation. The caps were made by breaking the needle from a discarded hypodermic needle and filling the perforation with paraffin. A 24 gage, 1-inch needle seems best suited for use in the test.

Readings, at the present time, are limited to a single one taken at the end of one hour, since additional information has not been obtained by the use of the curve plotted from the five-minute readings, nor any advantage observed in readings taken beyond the one-hour interval, as in the Westergren method.

The advantages of this simplified technic may be considered as follows: The elimination of one or more steps in the determination of the index materially reduces the time necessary for the procedure. The small amount of blood necessary (1.8 c.c.) allows the use of a small hypodermic needle, which causes a minimum of discomfort to the patient and facilitates entrance to the small veins of children. A minimum of equipment is necessary and, once the syringe (which can be put to other uses in the private office) is acquired, the apparatus can be found in any physician's office, with the exception of the

sterile citrate solution. The sterilization necessarily preparatory to the test is no more than is necessary for ordinary venipuncture. The index is determined by a single reading at the end of an hour, the apparatus requiring no attention during this time.

**MONILIASIS, Pulmonary, Bakst, H. J., Hazard, J. B., and Foley, J. A. J. A. M. A. 102: 1208, 1934.**

In three cases of monilia infection of the lungs, the organism was the primary agent in two and in one an invader secondary to *Bacillus tuberculosis*.

A marked variation in the clinical picture of pulmonary moniliasis was observed in the cases presented.

Excellent results were obtained with the use of iodides and with iodides supplemented by vaccine in the treatment of the cases of primary infection.

There are means of demonstrating the relationship of *Monilia* recovered from the sputum to the disease process in the patient. Agglutination reactions proved to be of uncertain value in the establishment of a diagnosis.

It is important to consider a diagnosis of pulmonary moniliasis in cases of unproved and atypical tuberculosis.

It is suggested that, in the wide group of cases clinically classified as chronic bronchitis, *Monilia* should be considered as one of the many possible etiologic agents.

**ALCOHOL, Specificity of the Test for, in Body Fluids, Heise, H. Am. J. Clin. Path. 4: 169, 1934.**

A simple and specific test for alcohol in body fluids is described below and its specificity has been demonstrated.

Specimens of blood and urine may be preserved for at least a month. For blood use sodium fluoride, and urine, benzoic acid.

The importance of the test lies in its ability to confirm a diagnosis of drunkenness for medicolegal purposes, as well as to give valuable information in differential diagnoses.

A preliminary survey of persons injured or killed in auto accidents suggests that alcohol may be a greater factor in such accidents than statistics indicate, and shows the importance of a nation-wide survey of the relationship of alcohol to automobile accidents.

The chemical test for alcohol in body fluids will be an important factor in arriving at conclusions concerning the intoxicating ability of certain beverages.

*Method.*—Distill mixture of 10 c.c. of urine with about 10 c.c. of half saturated picric acid containing about 10 per cent tartaric acid, collecting the first 10 c.c. of the distillate and mix. In separate tubes similar to those used for the standards, place 1 c.c. in one, and smaller measured amounts in the others, making the volume up to 1 c.c. in each case. Add 3 c.c. of the  $K_2Cr_2O_7$  reagent (0.33 per cent in 50 per cent sulphuric acid) to each tube, and place in boiling water bath four minutes. Compare colors with those of the standard scale. Divide the reading by the fraction of a cubic centimeter of distillate used, which gives the percentage of alcohol by weight. The use of several tubes permits close checking of the results and gives greater opportunity for having readings on the scale.

STRONG PERMANENT STANDARDS

BY WEIGHT PER CENT ALCOHOL	ALCOHOL BY WEIGHT	WATER C.C.
0.0		1.00
0.05	0.50 c.c. of 0.10 per cent	0.50
0.10	1.00 c.c. of 0.10 per cent	0.0
0.12	0.60 c.c. of 0.20 per cent	0.40
0.14	0.70 c.c. of 0.20 per cent	0.30
0.16	0.80 c.c. of 0.20 per cent	0.20
0.18	0.90 c.c. of 0.20 per cent	0.10
0.20	1.00 c.c. of 0.20 per cent	
0.22	0.73 c.c. of 0.30 per cent	0.27

If results are too low to be read, use the weak standards, using 2 c.c. of the distillate and known smaller amounts, bringing the volume to 2 c.c. in each case, add 1 c.c. of the reagent and place tubes in boiling water twelve minutes.

If blood is being tested take 2 c.c. of whole blood, plasma, or serum (all give the same results), add about 15 c.c. of the pierie-tartarie reagent, and collect the first 10 c.c. of the distillate. This is tested on the weak standard scale and the result multiplied by 5.

To each tube add 3 c.c. of  $N/15 K_2Cr_2O_7$  (0.33 per cent in 50 per cent  $H_2SO_4$ ). Place tubes in boiling water four minutes and seal.

#### WEAK PERMANENT STANDARDS

PER CENT ALCOHOL BY WEIGHT	ALCOHOL BY WEIGHT	WATER C.C.
0.0		2.0
0.005	1.0 c.c. of 0.01 per cent	1.0
0.010	2.0 c.c. of 0.01 per cent	0.0
0.013	0.52 c.c. of 0.05 per cent	1.48
0.016	0.64 c.c. of 0.05 per cent	1.36
0.019	0.76 c.c. of 0.05 per cent	1.24
0.022	0.88 c.c. of 0.05 per cent	1.12
0.025	1.00 c.c. of 0.05 per cent	1.00
0.028	1.12 c.c. of 0.05 per cent	0.88
0.031	1.24 c.c. of 0.05 per cent	0.76
0.034	1.36 c.c. of 0.05 per cent	0.64
0.037	1.48 c.c. of 0.05 per cent	0.52
0.040	1.60 c.c. of 0.05 per cent	0.40

Add 1 c.c.  $K_2Cr_2O_7$  reagent to each tube. Place tubes in boiling water-bath twelve minutes.

#### CANCER, Quantitative Modification of the Bendien Reaction in Sero-Diagnosis of Malignancy in Filipinos, Clemente, M. Bull. San Juan De Dios Hosp. 8: 120, 1933.

Blood from 55 cases recognized clinically as (a) clinically malignant, (b) clinically nonmalignant, and (c) normal has been tested by the modified Bendien reaction (Lowe). From the above study, the writer believes that:

The modified Bendien reaction can indicate any precancerous tendency of benign tumors. It can diagnose the presence or absence of malignant neoplasms.

It can prognosticate the progress of cancer cases after treatment.

The quantitative modified Bendien reaction is of value in the diagnosis of malignancy in both early and advanced cases.

Normal and nonmalignant serum give similar reactions with the test.

It compares favorably with other blood tests for malignancy.

#### RAT-BITE FEVER, Acquired From a Dog, Ripley, H. S., and Van Sant, H. M. J. A. M. A. 102: 1917, 1934.

Two cases are reported in which the animal vector was apparently the dog. The following comments are noted:

Kahn tests were strongly positive on both patients and the experimental dog that survived, while the Kolmer Wassermann reaction was usually negative or weakly positive.

A patient suffering with dementia paralytica was inoculated with the spirilla and developed a typical lesion and symptoms of rat-bite fever.

Flagella were demonstrated only with Burri's India ink method.

The mouse proved to be a better diagnostic animal than the guinea pig.

In one case, infection was contracted when no skin abrasion was noted, suggesting ease of penetration of the organism.

The clinical course of the disease showed marked variation.

A course of from three to six treatments with arsphenamine seems advisable.

## ABSTRACTS

**ALLERGY, Recognition of the Allergic State by Tissue Examination: The Respiratory Tract and the Nasal Sinuses, Steinberg, B. Am. J. Clin. Path. 4: 169, 1934.**

There is a distinct histopathological picture of the mucosa of the entire respiratory tract and of the accessory nasal sinuses associated with the allergic (atopic) state. The morbid changes are of a similar nature in the atopic conditions of asthma, hay fever and rhinitis. In asthma, in addition to the lungs, the rest of the respiratory tract including the nose and almost invariably the accessory sinuses show these morbid changes. This constant pathological picture of the respiratory, nasal and sinus mucosa permits recognition of the allergic (atopic) state involving these organs.

**SUMMARY OF THE ESSENTIAL CHANGES CHARACTERIZING THE HISTOPATHOLOGY IN ALLERGIC SINUSITIS AND HAY FEVER**

1. Edema.
2. Hyperplasia and hypersecretory activity of the goblet cells.
3. Thickening and hyalinization of the basement membrane.
4. Eosinophilic infiltration.
5. Hypertrophy and hypersecretory activity of the mucous glands.
6. Presence of mucus in lumen of sinus and glands.

**HISTOPATHOLOGY OF THE VARIOUS STAGES OF ALLERGIC SINUSITIS**

STAGE	MUCOUS GLANDS	EDEMA	GOBLET CELLS	BASEMENT MEMBRANE	AMOUNT OF MUCUS	EOSINO-PHILIA
Acute stage	Hyperplasia Hypertrophy Hypersecretory activity	Moderate to marked	Hyperplasia Hypersecretory activity	Slightly thickened, granular or homogeneous	Moderate to marked	75-90 per cent
Chronic stage	Hyperplasia Hypertrophy Hypersecretory activity and dilatation of glandular lumina	Moderate to marked	Hyperplasia Hypersecretory activity	Greatly thickened and homogeneous	Moderate to marked	35-90 per cent
Remission	Hyperplasia Little or no hypertrophy No secretory activity	Very slight	Not apparent	Moderately to greatly thickened and homogeneous	Little or none	15 per cent

**SUMMARY OF THE ESSENTIAL HISTOPATHOLOGICAL CHARACTERISTICS OF ALLERGIC (ATOPIC) MUCOUS MEMBRANES OF THE ENTIRE RESPIRATORY TRACT AND ACCESSORY NASAL SINUSES**

1. Hypertrophy and marked secretory activity of the mucous glands.
2. Presence of large amount of mucus in lumina.
3. Eosinophilia, from 15 to 90 per cent of all cells.
4. Edema of tissue.
5. Thickening and hyalinization of the basement membrane.
6. Hyperplasia of goblet cells with hypersecretory activity.

**CANCER, The Botelho Reaction as Modified by Itchikowa, Cruz, A. Bull. San Juan De Dios Hosp. 8: 95, 1934.**

This is but a preliminary report, hence the findings may still vary as the work is being continued. Basing from the above data, however, we can say that:

By the use of the modified method, the author was able to get 91.8 per cent positive results in cancer serum, as compared to 53.9 per cent positive results in the ordinary method.

False results are eliminated by the previous correction of the serum to be examined to conform with the normal serum.

The interpretation of results in the modified method is more accurate than the ordinary method, since there must be a persistent precipitate in positive cases, and the tube remains clear in negative cases.

The modified method is very sensitive for cases of carcinoma of the internal organs, especially carcinoma of the stomach.

The ordinary Botelho reaction is unreliable as a serodiagnosis for cancer because it gives a positive result when there is an excess of globulins in the serum, irrespective of whether the serum is cancer or not.

The percentage of albumin and globulin in cancer serum is variable. The albumin may be increased, decreased or normal; but the increase of globulin is found in those cases where there is a marked cellular disintegration in the tumoral mass or in those cases where the organism is greatly weakened as a result of the tumor.

Globulin is markedly increased in infectious diseases, in malnutrition, and in immunization.

**CANCER CELLS, In the Blood Stream, Pool, E. H., and Dunlop, G. R. Am. J. Cancer 21: 99, 1934.**

A large, hitherto undescribed cell was found in smears of the blood in 17 out of 40 cancer cases. Apparently the same cell was found in one noncancer case. The significance and origin of the cell are not established.

**POLYARTERITIS NODOSA, Haining, R. B., and Kimball, T. S. Am. J. Path. 10: 342, 1934.**

The term "periarteritis nodosa" does not accurately connote the morphological realities of the disease as we now know them. Dickson suggested "polyarteritis nodosa" as a name for this condition which seems a more descriptive term, free of misleading implications.

A specific filtrable virus with a selective affinity for the small and medium sized muscular type arteries of the body is probably the cause of polyarteritis nodosa. Any organ or combination of organs may be affected at any time in the course of the disease, and the resulting clinical manifestations may be bizarre in the extreme. The visceral arteries are involved more frequently than those of the extremities, and the organs most commonly affected are the kidneys, heart, gastrointestinal tract, pancreas, muscles, peripheral nerves, liver, spleen, and cerebrum.

Pathologically the inflammatory changes are not confined to the adventitia and periarterial connective tissue, as originally supposed. All the vascular coats are eventually involved and the primary changes take place in the media. Destruction of the media may give rise to aneurysm formation. Involvement of the intima with rupture of the elastic membrane may produce thrombosis. The process as a rule is progressive and in practically all of the reported cases there has been evidence of acute inflammatory changes superimposed upon the chronic reparative efforts. However, Arkin has described 1 case of histologic healing and he believes that in rare instances the process may come to a complete standstill.

Polyarteritis nodosa is seldom diagnosed or even suspected before autopsy, and even at autopsy there may be no gross indications of its presence. The internist should be familiar with the cardinal symptoms of the disease and its notoriously capricious behavior. Then, when the commoner possibilities have been carefully ruled out in a patient with septic manifestations and varied symptomatology, polyarteritis nodosa should be given consideration.

Carling and Hicks, and recently Schottstaedt have reported cases in which remission of symptoms seemed to follow the intravenous administration of arsenicals.

**PNEUMONIA, Lobar, Artificial Pneumothorax in the Treatment of,** Behrend, A., and Cowper, R. B. G. *J. A. M. A.* 102: 1908, 1934.

Eleven patients with unilateral lobar pneumonia were treated with artificial pneumothorax to compress the affected lung, with two deaths. Neither of these fatalities could be directly attributed to the pneumothorax.

The authors believe that collapse therapy is a rational form of treatment of lobar pneumonia, based on sound surgical principles.

That lung tissue affected by lobar pneumonia can be compressed by air has been shown clinically, by roentgenograms and at autopsy.

Artificial pneumothorax relieves the pain of pleurisy that frequently accompanies lobar pneumonia.

It is possible to induce a critical fall in temperature by artificial pneumothorax.

The authors believe that artificial pneumothorax is neither a "cure-all" nor a "therapia magna sterilisans," but it has shown itself to be a valuable adjunct in the treatment of lobar pneumonia and even, we feel, a life-saving measure in some cases.

The authors have seen no complications directly attributable to the procedure.

**ENDO MEDIUM, Can It Be Standardized?** Conn, H. J., and Darrow, M. A. *Stain Techn.* 9: 61, 1934.

The following formula is recommended: Dissolve, by boiling 5 gm. beef extract, 10 gm. peptone, and 30 gm. air-dried agar in 1,000 ml. distilled water. Adjust the reaction to about pH 7.4 and filter through cotton. Add 10 gm. lactose, and allow it to dissolve. Place in small flasks, 100 ml. to each and sterilize for fifteen minutes at 15 pounds' pressure. Just before use prepare a 1 per cent solution of basic fuchsin (of 85 to 90 per cent actual dye content) in 95 per cent ethyl alcohol. Add 1 ml. of this fuchsin solution to 100 ml. of the agar (melted); also 0.125 gm. anhydrous sodium sulphite dissolved in about 5 ml. distilled water. The medium should be light pink while hot and almost colorless after cooling.

**RELAPSING FEVER, In Children,** Varden, A. E. *Am. J. Dis. Child.* 48: 359, 1934.

Symptomatically relapsing fever as it occurs in California resembles the African form of the disease. The symptoms are much more violent than those of the European form, for in Europe the disease is mild unless complicated by typhus fever, which accounts for its high mortality in that area. In Africa the mortality is high probably because of complications and because the victims there are usually in poor health, while in California, even though the disease is usually fulminating, it is generally uncomplicated and its victims are persons in general good health. Prompt treatment is also a factor in the low mortality in California.

According to Toyoda, there is a bacteriologic relation between the European and the American type, and there is also a relation between the European and the African, but none between the American and the African variety. The European type is between the other two.

# The Journal of Laboratory and Clinical Medicine

VOL. 20

MAY, 1935

No. 8

Editor: WARREN T. VAUGHAN, M.D.

Richmond, Va.

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## EDITORIAL

### Creatine Metabolism with Especial Reference to Heart Disease

THE rôles of creatine and creatinine in the body economy have been subjected to much clinical, as well as fundamental, investigation in the past few years. The great interest in muscle metabolism was sharply accentuated by the fundamental contribution of Fiske and Subbarow,<sup>1</sup> who discovered in living voluntary muscle a labile compound of creatine and phosphoric acid which was hydrolyzed when the muscle was activated by stimulation and resynthesized when the muscle recovered at rest. At about the same time the Eggletons<sup>2</sup> reported the finding of a very unstable form of organic hexose phosphate, a phosphoric ester of glycogen or a precursor of both lactic acid and lactacidogen. This phosphagen played a rôle, they found, in the chemical mechanism of contractility, and it is now considered to be phosphocreatine. Fiske and Subbarow considered the phosphocreatine to consist of one molecule of creatine and one of phosphoric



acid and to exist in the muscle cell wholly as the secondary potassium salt. This is relatively stable in an alkaline medium, but hydrolyzes with increasing velocity as the hydrogen ion concentration rises. The relation of phosphocreatine to glycogen remains fairly constant, as both decrease under aerobic conditions, while in an anaerobiosis the phosphocreatine disappears more rapidly than the glycogen and therefore the ratio falls. Free creatine exists in the resting muscle to the extent of 20 per cent and increases threefold after fatiguing stimulation, according to Clarke and the Eggletons.<sup>3</sup>

Many other investigators have made important contributions to this subject. Vollmer<sup>4</sup> found phosphocreatine in much higher concentration in ventricular heart muscle than in the auricular muscle and it constitutes 75 per cent of the total creatine content of the heart. Pollack, Flack, Essex, and Bollman<sup>5</sup> found increasing values for phosphocreatine in the perfused dog hearts, in heart-lung preparations perfused with blood alone and with glucose and insulin added.

With the establishment of the important rôle of creatine in muscle metabolism, renewed interest in the origin of the substance has been manifested. The various amino acids, as the chemically related arginine and histidine, glycine, glutamic acid, and others have been tried with varying, but no unquestionable, success. Thus far no certain precursor of creatine has been experimentally established to the satisfaction of the accepted critics among the biochemists in this field. Many studies along these lines are being vigorously prosecuted.

#### CLINICAL METHODS OF STUDY

Evidence of disturbances in the creatine-creatinine metabolism may be obtained from carefully controlled studies of the blood and of the daily output in the urine. The modified Folin<sup>6</sup> method, which depends upon the Jaffe reddish brown color reaction of creatine to an alkalinized pure picric acid solution, is generally employed. One determination of the endogenous creatine in a 2 to 10 c.c. specimen is made and subtracted from the total creatine determined in an equal 2 to 10 c.c. specimen of urine in which the creatine has been transformed to creatinine by hydrochloric acid and autoclaving for fifteen minutes. A similar technique is used for determining creatine and creatinine in the blood filtrate and in a sulphuric acid digestate of wet muscle or tissue.

Creatine does not occur in the urine of normal, healthy, well-fed adults, but it is to be remembered that in the extremes of life (childhood and senescence), and in starvation, febrile states, acidosis, diabetes mellitus, exophthalmic goiters, liver diseases, muscular dystrophies, cancer, wasting diseases, and in pregnancy, the excretion of unstored or unconverted creatine occurs. Creatinuria is therefore significant only under controlled conditions and especially where there is an insured high carbohydrate intake.

Creatinine is a normal urinary waste product of endogenous protein metabolism. It is the converted anhydride of creatine. Creatinine elimination is more or less constant for the same individual in normal activity irrespective of fluid or food intake as long as these are adequate and balanced. The creatinine coefficient is the number of milligrams of creatine excreted in the urine in twenty-four hours per kilogram of body weight. In man this varies from 18 to 32, with an average of 25; in women, 9 to 26, with 18 as an average. On rest in

bed we have found in disease as others have noted in health that the creatinine output decreases sharply and almost disappears. No reason for this is apparent.

Blood creatine and creatinine studies are being more vigorously prosecuted now that the new interest has been aroused in the subject. Creatinine has long been recognized as an index of the grade of glomerular damage. There is some dispute as to the presence of these substances normally, but in spite of these, figures of 4 mg. per cent of creatine and 1 to 1.8 mg. per cent of creatinine are given and blood creatinine values above 2 mg. per cent are considered to indicate abnormal retention.

#### CLINICAL IMPLICATIONS

The reports of Brand<sup>7</sup> and his associates in patients with skeletal muscle dystrophies have given further impetus to human creatine-creatinine metabolic studies. In such patients there is either an excessive liberation of creatine, incomplete conversion into creatinine, or incomplete storage, and creatine administered or formed is eliminated in high percentage. In such experiments glycocoll (amino-acetic acid) was found to increase the creatinuria 40 per cent, with only slight urinary nitrogen rise. Alanine, sarcosine and arginine produced slight rises and betaine a temporary rise, while glutamic and nucleic acids, histidine, tyrosine and cystine were without influence. Others have confirmed these findings and added to them. Glycocoll has apparently produced chemical, as well as clinical, improvement in some muscular dystrophy patients. The increased creatinuria alone cannot be accepted as proof that glycocoll is a precursor; it may be primarily a mobilizer.

Creatinuria was found in most of our patients with acute coronary thrombosis and in a large percentage of the patients with acute congestive failure. This was considered to be significant and probably the result of the general anoxemia and the acidosis. Starvation may have been a factor, but a high carbohydrate cardiac diet failed to eliminate the creatinuria entirely. In most patients it gradually cleared up with rest in bed and digitalization. In some, glycocoll hastened the clearing of the urine of creatine. Under such circumstances it may have been the carbohydrate value that was the factor. In others, creatinuria persisted or cleared up for a day or two and reappeared. In a large series of patients followed day in and day out for months, the results show so many unexplainable irregularities that conclusions cannot be drawn.

The extensive studies of skeletal muscle chemistry have inaugurated analogous studies of heart muscle, for most students of cardiac disease have long felt that "failure" must be chemical rather than mechanical. The large red beefy heart that autopsy often reveals in hypertensive patients who have died in congestive failure cannot be satisfactorily explained on the mechanical basis. Cowan<sup>8</sup> has supplied evidence that supports the contention that chemical changes, in fact, creatine changes, may play an important rôle in failure of the heart. In his series of seventeen decompensated hearts, the creatine content averaged 147 mg. per cent moist weight (79.47 per cent water), 50 mg. or 25.8 per cent less than the values obtained in the analyses of a normal series of 48 hearts averaging 202 mg. per cent moist weight, while fifteen hearts from patients with miscellaneous causes of death contained 165 mg. per cent. Septicemia, sex and age showed no influence on the creatine content of heart muscle. Cowan also

showed that the cardiac hypertrophy induced by nutritional anemia was accompanied by increases of creatine with increase in muscle mass, but the mass increases faster than the creatine. He found, however, that thyroxin feeding increased the muscle mass, but resulted in a loss of creatine.

Seecof, Linegar and Myers<sup>9</sup> have demonstrated that the left heart has a higher content than the right ventricular wall. In 84 human hearts the mean was 188, the average 211 mg. per cent. The left ventricle averaged 21.9 per cent higher in creatine content than the right ventricle. Such studies as these strongly suggest that the functional capacity of the heart muscle is closely bound up with the total creatine content.

In a series of 12 dogs, kept on a creatine-creatinine-free diet for periods of several days to a week and showing no creatinuria, we<sup>10</sup> produced myocardial infarction by catheterization of the coronaries through the carotids and the injection of varying sized globules of mercury. These operations were carried out with a minimum of trauma, and the animals were fed a high carbohydrate solution by stomach tube in order to prevent starvation. A creatinuria invariably resulted in two or three days and lasted for about a week. Evidently the circulatory changes brought about in these experiments contributed toward the production of a creatinuria. Chemical analyses<sup>14</sup> of the infarcted and the normal heart muscle showed a sharp drop in glycogen in the infarcted muscle immediately following the infarction, apparently the result of the anoxemia. Edema detectable by a drop in total solids began to appear in the infarcted muscle within half an hour. The creatine loss in the infarcted muscle was slight until after the fifth hour of infarction after which diffusion from the damaged muscle was considerable and increased slowly. Analyses of infarcted human heart muscle showed similarly low creatine values.

In our further studies we<sup>11, 12</sup> made use of the isolated living rabbit heart and perfused it with an oxygenated Ringer-Locke solution in which the pH was maintained at about 8, the carbon dioxide being washed out by suction. To the perfusate various amino acids were added, as glycocoll and alanine in 100 mg. per cent concentration, creatine, arginine, methyl guanidine, aspartic acid, and glutamic acid in 10 mg. per cent solution. Experiments were continued for six to ten hours and the hearts, when they had failed and were just responsive to electrical stimulation, were cut down, divided and analyzed for total creatinine, most of which, of course, is creatine content.

A control series of hearts that had been perfused for a minute only, long enough to remove the blood, showed values from 144 to 161, with an average of 153 mg. per cent. The hearts perfused with the Ringer-Locke solution gave values of 123 mg. per cent, and most of the other amino acids gave only slightly higher values, some of which reached the control levels. Alanine and alanine-with-methyl-guanidine, however, gave total heart muscle creatine values distinctly above the controls. This work, of course, requires further confirmatory studies.

While matters are still in the experimental stage and nothing can be accepted as proved, nevertheless the field warrants further and intensive tilling. We may possibly be on the threshold of significant discoveries. At any rate the evidence



at hand is sufficient to justify the conclusion that chemical factors, most likely those concerned with creatine as well as glycogen metabolism, probably play an important rôle in cardiac failure.

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—G. H.

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#### Item

#### American Society of Clinical Laboratory Technicians

The third annual convention of the American Society of Clinical Laboratory Technicians will be held at Haddon Hall, Atlantic City, N. J., June 10, 11, and 12. An unusually interesting program has been arranged and all clinical laboratory technicians, whether members of the society or not, are cordially invited to be present.

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#### Errata

On page 708 of the April issue, the author's name should read: "James J. Short, M.D., F.A.C.P., New York, N. Y.," and the first paragraph of the footnote should read: "From the Laboratories of the Life Extension Institute, New York," omitting the remainder of the sentence.





(MORGAN)

*J. P. Morgan.*

# *The Journal of Laboratory and Clinical Medicine*

Vol. 20

JUNE, 1935

No. 9

## CLINICAL AND EXPERIMENTAL

### OBSERVATIONS ON THE AUTONOMIC CONTROL OF CARDIAC VASCULATURE\*

#### ATTEMPTED VAGUS AND SYMPATHETIC OVERACTION ON CAT'S HEART

HAROLD F. ROBERTSON, M.D., AND ARTHUR J. DERBYSHIRE, A.B.,  
BOSTON, MASS.

SEVERAL investigators have demonstrated that it is possible to anastomose a somatic motor nerve to an autonomic nerve and have the somatic fibers grow into the autonomic sheath.<sup>1, 2</sup> That such growth occurred was shown by physiologic responses in the organs connected with the affected innervation when it was stimulated, and after the death of the animal by histologic examination. In only one series of experiments however were spontaneous anatomic and physiologic changes produced. Cannon anastomosed the caudal end of the cut phrenic nerve of the cat to the cephalic end of the cut cervical sympathetic cord.<sup>2</sup> Changes developed similar to hyperthyroidism presumably by overaction of the sympathetic end-organs in the thyroid gland.

The object in our experiments was to test the ability of similar nerve anastomoses to activate appropriate end-organs when phrenic impulses and phrenic fibers could be shown conclusively to have extended toward the region of the involved end-organs or ganglia. To determine that the phrenic fibers had grown toward these regions we tested the nerves with modern electrical apparatus for the electrical phenomena that accompany their nerve impulses, and later checked these findings by histologic study. In the event of signs of activity developing we planned to produce at will certain changes in the organs affected and to alter

\*From The Laboratory of Surgical Research and Laboratory of Physiology, Harvard Medical School.

Received for publication, September 25, 1934.



their activity by appropriate sutures. Because of our interest in cardiac circulation we chose the cardiac autonomic innervation as the field for experimentation. We hoped, at least, to cause tachycardia with vascular dilatation of cardiac vessels by phrenic-sympathetic anastomoses, and bradycardia with vasoconstriction of cardiac vessels by phrenic-vagus anastomoses.

Besides the evidence presented by Cannon there have been, from time to time, reports on the effects of sympathetic overstimulation produced however by means other than nerve anastomoses. Von Beehterew, among others, demonstrated that sympathetic irritation led to vascular spasm and endarteritic changes in the region supplied by the affected nerves.<sup>3, 4</sup>

Recently Adamson and Aird, by cutting the sacral outflow in cats, produced changes in the bowel similar to Hirschsprung's disease, the result apparently of unopposed sympathetic activity.<sup>5</sup> Though investigators have cut one or other, or both, the autonomic outflows to the heart with no noticeable pathologic change in the heart muscle, and no change in the vasculature, we repeated this procedure in some cats. We also, in some of the nerve suture experiments, cut the antagonistic nerve supply to the heart on the grounds that any effects of overstimulation would be amplified.

#### METHOD

Our preparations were as follows: In a series of cats unilateral or bilateral anastomosis was done between the cephalic end of the cut phrenic nerve, or one of its roots, and the caudal end of either the sectioned cervical sympathetic trunk or the sectioned vagus nerve or the cephalic end of the cut thoracic sympathetic chain. The opposing innervation to the heart was eliminated in a few cases. In both the case of the caudal end of the cut vagus nerve and the cephalic end of the cut thoracic sympathetic chain, most of the fibers are cut distal to their nuclei, and therefore degenerate since they are connector or preganglionic fibers with nuclei in the brain stem and cord respectively. This leaves room for ingrowing anastomosed fibers. In the case of the caudal end of the cut cervical sympathetic cord, however, the fibers are mostly postganglionic or effector with nuclei in the stellate ganglion and survive leaving, in theory, no opportunity for the ingrowth of phrenic fibers. Moreover it has been held that anastomoses should be made only to autonomic nerves containing a preponderance of preganglionic fibers such as the vagus nerve contains, rather than to nerves with a majority of postganglionic fibers as in the cervical sympathetic cord.<sup>6</sup> The nerve sutures were done with a fine eye needle and the finest silk thread. No suture was done under tension.

In other cats unilateral or bilateral division of either or both the sympathetic or parasympathetic innervation to the heart was carried out, the principles laid down by Cannon being followed with care so that all fibers to the heart were actually divided.<sup>7</sup>

The preparations thus secured were studied by taking frequent pulse counts, and watching cardiac reaction to fear, rage, atropine, and to asphyxia.<sup>7</sup> One might have expected, if the vagus end-organs in the heart were stimulated by anastomosis of the vagus to a rhythmically discharging nerve such as the phrenic,

that the heart would be slowed rhythmically or permanently and that fear and rage would have little speeding effect on the rate while atropine would allow the heart to speed up especially if adrenaline secretion were stimulated by fright or rage. Asphyxia from its earliest stages should greatly slow the heart but atropine should allow escape from this effect or prevent it. Conversely if the sympathetic end-organs of the heart were stimulated by phrenic impulses the heart should be more rapid than usual, fear and rage should increase the rate of beat and atropine should allow still further increase in rate. Asphyxia should greatly speed the heart, atropine perhaps accelerating the effect. In those cats where, besides nerve suture, denervation of the opposing system was carried out, inhibitory or stimulatory effects of the excised system on the heart's reaction to phrenic stimulation would of course be lacking. In the group that had the parasympathetic system, the sympathetic system or both systems removed reactions to fear or rage and atropine were useful in determining the presence or absence of fibers of either system. Whenever the chest was opened the heart was usually thoroughly inspected. The progress of the growing nerve anastomoses was followed by exposing the vagus or sympathetic nerves in either the neck or the chest and testing them for phrenic nerve impulses by a special technique which we describe under our observations.

#### OBSERVATIONS

To follow the progress of the anastomosed nerves along their new sheaths we used a voltage amplifier called the "Neurophone" (Fig. 1). It was possible by this means to detect phrenic nerve impulses without disturbing the nerve or its sheaths. The neurophone consists of four stages of transformer coupled amplification that are run by batteries contained in the same steel case. The output of this amplifier feeds two more stages of amplification and a loud-speaker in a separate steel case for audibly detecting the responses. This power stage can run on either alternating current or direct current. These two portable units were built by Mr. E. L. Gareau, in the Laboratory of Physiology, for use in operating rooms to examine the action potentials of nerves and muscles.<sup>8</sup> The amplifier can detect inputs of about three microvolts.

For the particular purpose of recording the action potentials of the phrenic nerve or the potentials of phrenic fibers that had regenerated into the sheath of either the sympathetic or the vagus nerves in the neck or chest, it was necessary to combine high sensitivity with good shielding. Sensitivity was necessary in order that impulses might be recorded from fibers with small diameters, such as those of regenerated nerves, since the action potential is proportional to the cross-section of the nerve fiber, and in order that the sheath around the nerve would not have to be removed or disturbed with consequent interference with the regeneration of the nerve fibers.<sup>9</sup> From a series of acute preparations of the cat's phrenic nerve it was found that the farther apart the ground lead and the differentiated lead were placed, up to 1 cm., the better were the responses obtained. In the chest it was found that the E. K. G. was the main source of interference and an adequate shield from this disturbance had to be provided. In the neck as well as in the chest the spread of the action potentials of the muscles of

respiration was a confusing component that had to be completely eliminated if definite results were to be obtained.

We were finally able to avoid these forms of interference and still obtain enough sensitivity by the use of a special type of electrode suggested to us by Dr. Hallowell Davis (Fig. 2, 1). This consisted of a thin silver plate about 6 mm. wide that was bent in the shape of the letter J. The inner side of the curve was insulated with a baking varnish, and a piece of silver wire placed on this varnished surface was baked in place. The silver wire was connected by an adapter to the grid of the first tube of the neurophone through a sterilizable shielded cable. The shield of this cable was continuous with the flat silver plate of the electrode and was led to the ground of the amplifier. The baking varnish was scraped

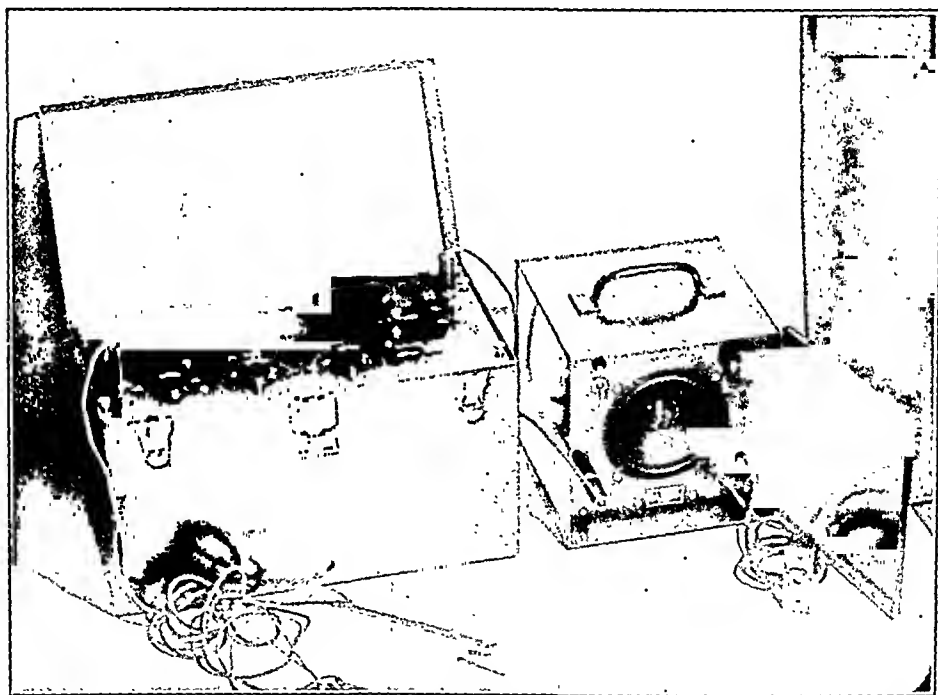


Fig. 1.—The neurophone. Input and amplifying circuit, left; loud-speaker, right; sterilizable cable and electrodes in foreground.

away from the outer edges of the bent silver plate and from the silver wire in the center. The electrodes thus prepared were sterilizable. The exposed nerve with its sheath intact could be placed upon this electrode without interference with either its sheath or its connections. The nerve made three contacts; the first one to the silver plate; the second to the differentiated lead to the grid of the first tube; and the last to the silver plate again. Extraneous electrical disturbances which travel along the surface of nerves, such as the E. K. G. and the spread of the action potentials originating in the respiratory muscles, were shunted through this ground plate and not recorded. The potentials developed by the nerve fibers, however, were recorded as differences of potential between the silver wire in the middle of the electrode and the points of the nerve touching the silver plate.

The proof that this electrode was reliably shielded depended upon many cases in the normal cat in which nothing was obtained from the normal sympathetic nerves in the neighborhood nor from a strip of connective tissue that was left connected to the animal body, at both ends, and yet the phrenic nerve gave audible responses correlated with respiration. Moreover similar tests were carried out in the cats in which the phrenic nerve had been anastomosed to the sympathetic or vagus nerve. In these too we found that extraneous impulses were not reproduced by our amplifier. The fact that impulses, synchronous with respiratory and cardiac rhythms, have been demonstrated in sympathetic and vagus fibers coursing toward the heart was kept in mind.<sup>10</sup> In several acute experiments on cats we found that the neurophone did not pick up such impulses though at the same time the characteristic normal phrenic impulses were heard very well. Impulses were also detected in the normal vagus nerve during the inspiratory phase of artificial respiration especially if excess inflation occurred. The same impulses were not heard during spontaneous respiration. They

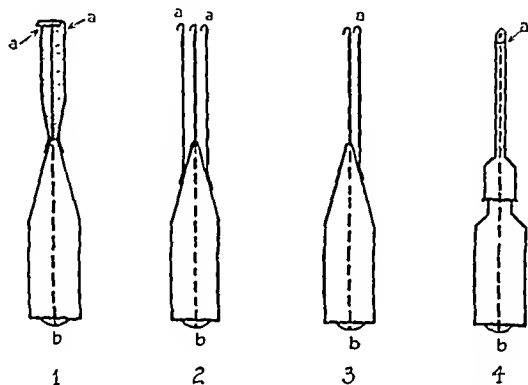


Fig. 2.—Electrodes used with the neurophone. a, Ground lead; b, differentiated lead (dotted line). 1, Moderate sensitivity, excellent shielding and selectivity. This electrode was found best for our purpose. 2, Very sensitive, good shielding and selectivity, but hard to place on the nerve. 3, Fairly sensitive, poor shielding. 4, Shielding and selectivity good, but needle could not be forced into small nerves well.

were interpreted as afferent vagus impulses arising from the lung and respiratory passages. We were certain that impulses other than those of the phrenic nerve, but perhaps capable of resembling them, would not confuse our observations. In numerous instances, when typical phrenic impulses were found, both above and below the level of the nerve suture in such a preparation, histologic examination confirmed the presence of healthy nerve fibers growing in the anastomosed nerve sheaths.

We tried other forms of electrodes but for various reasons they were found inadequate. These electrodes are all shown in Fig. 2. The electrode shown in Fig. 2, 2 was found to be sensitive and to shield out the extraneous activity but the wires were too flexible and were hard to place under delicate nerves. The electrode in Fig. 2, 3 lacked good shielding but was good in sensitivity. Due to the amount of E. K. G. and muscle action potentials picked up this electrode was discarded. Finally the fourth type gave so much selectivity and shielding that the phenomena of the nerve could not be detected. This electrode could

only be used if it was introduced into the nerve through the sheath. This produced so much damage that it was not serviceable.

We can summarize our technical procedure by describing the limitations of the method. The noise level of the amplifier at about  $2\mu$  determined the limit of the overall sensitivity that could be obtained. The potential changes detected from regenerated nerves are small because first, the potential developed is directly proportional to the cross-section of each fiber, and second, the sheath of the nerve shunts much of the potential change. These conditions require sensitivity of detection. Finally we could not use as great a separation of ground and differentiated leads as desirable since it was not always convenient to expose the required length of nerve. The method is not as sensitive as desired, but for the present we are content with being certain that such responses as we have obtained are definitely those of the nerve fibers in question.

With such a technic we have little difficulty in deciding whether certain portions of nerve sheath contained phrenic impulses. Six animals in which nerve suture was done gave positive evidence of successful anastomoses. Care had to be taken during tests that the nerve was not subjected to tension and that the sheath was not traumatized, otherwise the nerve might be thrown into a state of temporary block and no result obtained. In several animals with phrenic nerve anastomosed to vagus, normal phrenic impulses were picked up as far caudally as the aortic arch, and in one cat with a phrenic sympathetic suture phrenic impulses were followed along the cardiac fibers from the stellate ganglion toward the heart. Histologic section as mentioned above confirmed electrical findings in many instances.

Though some of the cats have now lived for over a year with successful nerve anastomoses no gross anatomic change has taken place.\* In numerous autopsies on cats with either successful nerve sutures or on cats whose cardiac innervation had been interfered with by some other method no histologic change was noted.

In the cats with nerve anastomoses and in those with section of some part of the cardiac innervation there was no physiologic evidence that sympathetic or parasympathetic end-organs were overactive. Fear or rage, and atropine, in the case of the simple denervation experiments produced no unusual reactions other than those already outlined by Cannon and others as seen when vagus, sympathetic or complete cardiac denervation is done.<sup>7</sup> In the cats with nerve anastomoses alone or anastomoses in conjunction with denervation of the opposing system no evidence of sympathetic or parasympathetic overaction was found by the production of fear or rage, asphyxia, or the use of atropine, and so, although phrenic fibers had undoubtedly grown into the vagus and sympathetic sheaths, no effect on the respective end-organs was discovered by the physiologic tests employed.

\*Two cats with successful phrenic-vagus anastomoses and one cat with successful phrenic-sympathetic anastomosis have (at time of publication) now been observed for over two years. In all three phrenic impulses could be traced electrically as far caudally as the aortic arch along the anastomosed vagus or cardiac sympathetic nerve sheaths. There is no evidence of anatomic or physiologic change in the hearts of these animals.

## SUMMARY

In cats anastomoses between the phrenic and the vagus or the sympathetic nerves were done unilaterally or bilaterally and by electrical tests active phrenic fibers were shown to have grown past the point of anastomosis toward the heart. The electrical findings were confirmed by histologic studies. Though no effects on appropriate end-organs and associated viscera have been demonstrated, it must be remembered that in previous reports the commencement of such effects was often delayed well over a year. Therefore such cats as we have left are being carefully followed in the hope that later on effects will be established.\* Our ineffectual efforts to create anatomic changes in the heart and its vessels by section of the sympathetic or parasympathetic cardiac fibers in an attempt to produce autonomic imbalance, correspond with previous observations in similar procedures on the heart. The technique of testing our nerve preparations provided a good example of what may be done in the field of neurosurgery with modern electrical apparatus; it is likely in the future to undergo still further development to the advantage of those who wish to define nerve tracts and study their activity.

We wish to thank Dr. Hallowell Davis whose suggestions concerning the electrodes and the amplifier have been indispensable.

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\*See footnote on page 900.

# THE VALUE OF THE TAKATA AND ARA REACTION AS A DIAGNOSTIC AND PROGNOSTIC AID IN CIRRHOSIS OF THE LIVER\*

ALEX B. RAGINS, M.D., CHICAGO, ILL.

## INTRODUCTION

THE mercury sublimate, diamond fuchsin test was first introduced in 1925 by Takata<sup>1</sup> as a laboratory test for differentiation between lobar pneumonia and bronchopneumonia. That same year Takata and Ara,<sup>2</sup> working on spinal fluid, introduced a modification of the test, thus affording a differentiation between the syphilitic and meningitic types of spinal fluids. Many German investigators<sup>3</sup> utilized the test for the determination of syphilitic meningitis with fairly good results. In this country, Monias,<sup>4</sup> in 1928, introduced the Takata and Ara test, commenting favorably as to its accuracy and simplicity.

Staub<sup>5</sup> mentioned the application of this test as an aid in the diagnosis of liver cirrhosis. In 1929, Jezler<sup>6</sup> utilized this test on blood serum and found that it would produce a flocculation in a great majority of instances where patients were suffering from cirrhosis of the liver. Jezler<sup>7</sup> again successfully carried out the test but with more detail and with some modification. Other investigators, Oliva and Pescoroma,<sup>8</sup> Skouge,<sup>9</sup> Crane,<sup>10</sup> and Hugonot and Sohie,<sup>11</sup> tried it with convincing findings.

## METHOD

During the last year I have tried the test on 276 patients, using the Jezler modification which is as follows. The reagents required are a 0.9 per cent solution of sodium chloride, a 10 per cent solution of sodium carbonate, a 0.5 per cent solution of mercuric chloride, and a 0.02 per cent solution of diamond fuchsin. All these solutions are made with double distilled water.

A series of eight Wassermann tubes of the same diameter, rinsed in double distilled water and carefully dried, and each containing 1 c.c. of a 0.9 per cent saline solution, are set up. Then 1 c.c. of blood serum (nonhemolyzed and not more than twenty-four hours old) is added to the first tube. After the contents of this tube are well mixed 1 c.c. is pipetted off and placed in the second tube. Then, 1 c.c. is withdrawn from the second tube and placed into the third. This procedure is repeated with the remaining tubes, and the last cubic centimeter is discarded. The final set-up then consists of eight tubes containing serum in the dilutions from 1:2 to 1:256. To each of the tubes 0.25 c.c. of a 10 per cent solution of sodium carbonate is added, and the contents are well shaken. Immediately afterward 0.3 c.c. of a freshly prepared Takata and Ara reagent,

\*From the Department of Pathology, Cook County Hospital, Dr. R. H. Jaffé, Director.  
Received for publication, September 26, 1934.

containing equal parts of a 0.5 per cent mercuric chloride and 0.02 per cent solution of diamond fuchsin, are added to each tube, and the rack is shaken thoroughly. This completes the procedure.

Readings are made immediately after, then one-half hour after, and twelve to twenty-four hours after the completion of the test. A positive Takata and Ara reaction is characterized by a flocculation occurring in the tubes having a dilution from 1:16 through 1:64; that is, flocculation must occur in at least these three tubes in order to have this considered a positive test. Jezler<sup>7</sup> claims that the strength of the reaction occurs at the time of flocculation. A three-plus T. A. R.\* is present when flocculation occurs immediately, a two-plus T. A. R. is present when flocculation occurs one-half hour after the test is carried out, and a one-plus T. A. R. presents itself when flocculation occurs twelve to twenty-four hours after completion of the test. Plus-minus reactions are considered when questionable flocculations occur after twenty-four hours. In my series of cases, however, I found flocculation occurring immediately after completion of

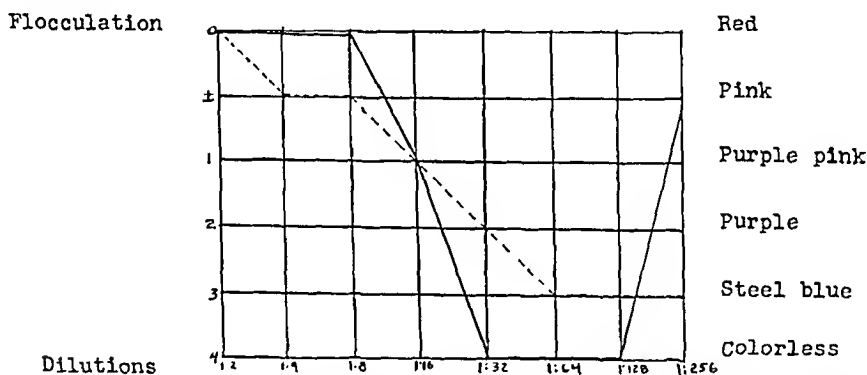


Fig. 1.—Dotted line shows color changes in a normal serum. Solid line shows flocculation curve in a positive T. A. R. serum.

the test in all my positive cases, with very little, if any, change in the flocculation after twelve to twenty-four hours, and I was led, therefore, to classify the strength of the T. A. R. on the basis of four-plus, three-plus, two-plus, one-plus, plus-minus, and negative, depending upon the heaviness and amount of the flocculations that occurred.

The colors produced in a series of test tubes, especially in the negative T. A. R., are invariably constant. The first tube has a red to a reddish pink color. The second and third tubes are pink; the fourth, purplish pink; the fifth, purple; and the sixth, seventh, and eighth tubes, steel blue (see Fig. 1).

Nicole<sup>12</sup> has shown that blood serums will persistently give this curve, and even in spinal fluid where blood serum is present such a curve will manifest itself. This curve, according to Nicole, is due to the albumin concentration in the serum.

## RESULTS

In this series of 276 cases, the clinical diagnosis was used as a basis for classification of the diseases and wherever possible was substantiated by post-

\*T. A. R., the abbreviated form of Takata and Ara reaction.



mortem examination or surgical biopsy. The cases were classified into fourteen main groups (Table I), with cirrhosis of the liver heading the list.

Of fifty-nine cases\* diagnosed clinically as cirrhosis of the liver, forty-five cases gave a four-plus T. A. R. in the blood serum, four cases gave a three-plus T. A. R., three cases a two-plus T. A. R., and two cases a one-plus T. A. R. Three cases gave a plus-minus T. A. R. and two were negative.

In this group twelve of the cases were confirmed by postmortem examination or surgical biopsy of the liver. In one case, with a four-plus positive T. A. R., an exploratory operation of the abdomen was performed, and a large quantity of serosanguineous fluid was found. The liver, spleen, and kidneys were found to be grossly normal. Unfortunately, a biopsy of the liver was not taken, thus making impossible absolute proof of a cirrhotic process of the liver in this patient. In two of our autopsied cases primary carcinoma of the liver was found to be associated with cirrhosis of the liver.

In some cases of cirrhosis of the liver additional T. A. R. tests were carried out in ascitic fluid and pericardial fluid. With one exception, these were found to be positive. Ten cases of ascitic fluid were tested, nine of which gave a positive T. A. R. and one a negative T. A. R. One of pleural fluid gave a positive T. A. R.

As to the number of cases in the cirrhosis series with a positive T. A. R. that showed evidence of hepatic decompensation, 46 are enumerated in Table I. One case had ascites with a negative T. A. R.

Chronic consumption of alcohol was admitted in 43 cases with a positive reaction and in two patients with a negative reaction. Of the remaining thirteen cases a history of alcoholism was either not obtained or denied.

As to the mortality rate, 32 patients who had had a positive T. A. R. had died during the course of observation, a period of ten months. Two positive T. A. R. cases were sent home, and the eight remaining cases of the series are still in the hospital at this writing.

Other tests were run wherever possible, but although not in any manner complete, are still sufficient to compare with the T. A. R. Of all tests in cases of liver cirrhosis, the icteric index showed a marked parallelism with the T. A. R. Thirty positive cases showed an increased icteric index; four positive cases were normal. One negative case showed a high icteric index. As to the urea nitrogen of the blood serum in these cases, twelve positive T. A. R. cases showed an increase, and nineteen positive cases were normal, thus denoting that urea nitrogen is not a valuable adjunct in indicating liver damage. Of the two negative cases one showed an increase in urea nitrogen, and one was normal.

Of the fourteen cases of acute hepatic insufficiency other than cirrhosis of the liver, there were three cases with a three-plus positive T. A. R., and two cases with a plus-minus positive T. A. R. All these five patients improved and were sent home. Two of the three-plus cases were patients suffering from acute hepatitis and one from acute yellow atrophy. The two plus-minus positive cases were patients with acute hepatitis.

\*In five cases T. A. R. tests were carried out on postmortem serum, and the reactions were no different from that of the serum of living patients. Skouge<sup>8</sup> has shown that tests in postmortem serum can be successfully carried out, provided the blood is not hemolyzed and the serum does not contain too much fat.

TABLE I

CLINICAL DIAGNOSIS	NO. OF CASES	T.A.R. OF BLOOD SERUM AND ASCITIC* AND PLEURAL† FLUIDS		P. M. OR SURGICAL FIND. IN POS. T.A.R.		P. M. OR SURGICAL FIND. IN NEG. T.A.R.		HISTORY OF ALCOHOL- ISM		ASCITES		UREA NITROGEN IN POSITIVE T.A.R.		UREA NITROGEN IN NEGATIVE T.A.R.		ICTERIC INDEX IN POS. T.A.R.		ICTERIC INDEX IN NEG. T.A.R.	
		POSITIVE	NEGATIVE	CONF. DIAG.	DIF. DIAG.	CONF. DIAG.	DIF. DIAG.	POS. T.A.R.	NEG. T.A.R.	POS. T.A.R.	NEG. T.A.R.	INCREASED	NORMAL	INCREASED	NORMAL	INCREASED	NORMAL	INCREASED	NORMAL
Cirrhosis of liver	59	57	2	12	1	1		44	2	46	1	12	19	1	1	4	30	1	8
Acute hepatic insufficiency other than cirrhosis of the liver	14	5	9			1		1	5			2		1	1		3		
Hepatitis due to arsenical and industrial poisonings	3	1	2						1						1	1	1	2	
Malignant tumors without liver metastases	7	3	4	2		2		2	1	1	1	1		3	1	1	1	2	
Malignant tumors with liver metastases and primary tu- mors of livers	20	11	9	1		5		4	7	5	2	3	2	7	3	3	2	5	
Normal pregnancies	10		10																
Toxemias of pregnancy	6	3	3			1		1	7	1	4	1		1	7			3	
Cardiovascular renal disease other than syphilis	18	2	16			1									3			2	
Diseases of the kidney	11	1	10			2		7	3	3	4	4		9	1		1	1	
Acute, subacute, and chronic in- fections	54	12	42			3			7	2	4			8	7		2	2	
Blood dyscrasias	10	1	9	1		2			1	1	1			2		1		1	
Diseases of the glands of internal secretion	16	8	8			2								3					
Syphilis	4	1	3	1		7		6	10	1		2	1	3	3	1	2	1	1
Varicella	44	9	35					64	45	57	16	25	27	46	21	8	40	3	3
Total	276	114	162	17	2	26		64	45	57	16	25	27	46	21	8	40	8	29

\*Four cases of ascitic fluid tested.

†One case of pleural fluid tested.

Skouge<sup>9</sup> speaks of several cases of acute hepatic insufficiency which had positive T. A. R.'s during the active stage, and these positive reactions were negative when their conditions improved. The remaining nine cases were those of acute hepatitis, and all were negative. One of the two negative cases that died was autopsied. This case also gave a negative T. A. R. on the postmortem blood serum. At autopsy there was evidence of a severe acute hepatitis.

In eleven cases of this series, the icterus index was high, and of the remaining three no laboratory data were given.

In three cases of liver damage due to arsenical or industrial poisonings, only one case gave a strongly positive reaction. This man was being treated for syphilis two years after the infection, and following the first injection of arsphenamine, he developed a diffuse erythema, which was at first diagnosed as scarlet fever, but the erythema was soon replaced by a marked jaundice and coma. At that time his T. A. R. was a decided four-plus. At a later date, it was still a strong four-plus. The patient has had several relapses associated with small gastric hemorrhages and is at present in a poor state, despite the fact that he has released himself from the hospital.

There were seven cases of malignant tumors without metastases to the liver, three of which showed a positive T. A. R. Of these three positive cases, there was one four-plus T. A. R. in a carcinoma of the hepatic duct, associated with an obstructive biliary cirrhosis. This patient, a Japanese, gave a history of drinking a quart of alcohol almost daily for forty years. The second case was a carcinoma of the stomach, and the third manifested itself as a carcinoma of the prostate and was proved to be so by surgical biopsy. The patient died soon after operation, and a postmortem examination was not permitted. The remaining four cases were negative and consisted of one case of carcinoma of the stomach, one a carcinoma of the rectum, a third was diagnosed upon laparotomy as a carcinoma of the head of the pancreas and the fourth case was a carcinoma of the common duct, with marked evidence of biliary obstruction, as was pointed out at autopsy.

In the group of carcinomas with metastases to the liver and primary carcinomas of the liver, there is a total of twenty cases. Eleven cases showed evidence of liver damage, as indicated by a positive T. A. R. In the latter group, I found two carcinomas of the stomach to have a four-plus T. A. R. Two had a two-plus T. A. R., and two had a one-plus T. A. R. Three had a negative T. A. R.

Of the carcinomas of the head of the pancreas, the T. A. R. was negative in the only two cases which I had. In carcinoma of the extrabiliary tract, excluding carcinoma of the gallbladder, two cases were negative and one gave a plus-minus reaction, the latter test being run on the ascitic fluid.

Of three cases diagnosed as primary carcinoma of the liver, two were three-plus, and one was negative, the latter one proving to be a carcinoma of the liver, upon surgical biopsy of both the liver and a mesenteric lymph node.

In one case of neurocytoma with a negative T. A. R., the clinical diagnosis was substantiated by postmortem examination. In another case, in which the primary tumor was undiagnosed, there was a marked obstruction of the inferior vena cava. The T. A. R. on the serum was negative. In this group, the clinical diagnosis of six cases was confirmed by postmortem examination or surgery.

In a series of ten normal pregnancies, the T. A. R. was found to be negative in all cases. They subsequently delivered normally without any complications.

Six cases of toxemia of pregnancy were studied. In this series there were three cases which showed evidence of a positive T. A. R. In one case of pregnancy associated with hypertension the T. A. R. was two-plus, and in two cases of eclampsia the T. A. R. was one-plus. All three patients improved under proper management and left the hospital in better health than upon admission.

Of the remaining three cases, one, an eclamptic, gave a negative T. A. R. and at postmortem examination gave the findings typical of eclampsia. The other two cases which gave a negative T. A. R. were pregnancies associated with hypertension.

In a group of cardiovascular renal diseases other than syphilis eighteen cases were studied, of which only two cases gave a positive T. A. R. One was a case of cerebral hemorrhage and gave a four-plus reaction. The other was a case of arteriosclerotic heart disease, giving a one-plus T. A. R. Of the remaining sixteen cases, two were hemiplegias, one of which at postmortem examination showed the essential findings of a ruptured aneurysm of the anterior communicating artery. As an incidental finding there was an echinococcus simplex cyst in the right lobe of the liver. Four patients suffered from arteriosclerotic heart disease, nine patients were victims of hypertensive heart disease, and one case was considered a primary myofibrosis. All these heart patients were in a state of cardiac decompensation upon admission to the hospital, and when the test was carried out on them, four patients had marked ascites, and the others had edema of the lower extremities associated with dyspnea.

Of eleven cases classified as diseases of the kidney, only one case gave a plus-minus T. A. R., whereas all the others were negative. The single positive case was that of a bilateral hydronephrosis. The others consisted of two cases of chronic glomerulonephritis, one of a unilateral polycystic kidney, one of lipid nephrosis, one of pyelonephritis, and five of malignant nephrosclerosis. The clinical diagnoses of two of these cases were corroborated at autopsy.

Of 54 cases classified under acute, subacute, and chronic infections, four cases gave a four-plus positive T. A. R. Two patients were suffering from acute rheumatic heart disease, both dying later. A third case was a far-advanced pulmonary tuberculosis and the patient had a history of drinking one quart of alcohol daily for five years. In the fourth case, the patient came into the hospital with lobar pneumonia, and after a stormy course recovered. Upon his recovery, it was found that he was an habitual drunkard and moderately demented.

One of the three cases having a three-plus T. A. R. was an acute rheumatic heart patient who later improved and went home. The second was a case of lobar pneumonia, and the third was a case of tuberculous pleurisy with effusion. The patient gave a history of drinking at least one pint of alcohol daily for the last sixteen years.

A one-plus T. A. R. was obtained in a case of pulmonary tuberculosis and in a case of lobar pneumonia.

In this series of infectious diseases, eleven cases of pulmonary tuberculosis, one case of gonorrheal arthritis, two of malignant endocarditis, five of bronchopneumonia, one of acute follicular tonsillitis, one of typhoid fever, one of acute articular rheumatism, twelve of lobar pneumonia, one of tuberculous polyserositis, one case of septic sore throat, one of postpneumonic empyema, one of tuberculous pleurisy with effusion, and one of septic meningitis, gave negative T. A. R.'s.

In ten cases of blood dyscrasia, only one case gave a positive T. A. R., the reaction being two-plus. This was a case of acute stem cell leucemia in an aleucemic state, associated with a septicemia due to a hemolytic streptococcus. The liver in this case showed, microscopically, a dense infiltration of undifferentiated cells in the portobiliary septa, as well as in the capillaries, and in addition to these, the liver cells were slightly displaced by intralobular cell accumulations.

The nine remaining cases of blood dyscrasia were negative. One was a case of acute stem cell leucemia, four were that of pernicious anemia, one an aplastic anemia, two were splenic anemias, and one was a case of aleucemic myelosis. In two instances postmortem examination confirmed the diagnoses.

As to the diseases of the glands of internal secretion and the response of their serum to the T. A. R., sixteen cases were studied. Eight cases showed a positive T. A. R. Two cases of exophthalmic goiter and one case of toxic adenoma gave a four-plus T. A. R. One case of toxic adenoma was a three-plus T. A. R., and one case of myxedema associated with a pituitary disturbance, as indicated by clinical evidence of acromegaly, gave a two-plus T. A. R. Another case of myxedema gave a one-plus T. A. R., and two cases of exophthalmic goiter gave a one-plus T. A. R. Of the remaining eight cases, seven exophthalmic goiters were negative, and one toxic adenoma was negative.

Four cases of syphilis were studied. All patients were negative except one, who had a very much enlarged liver, and upon laparotomy and biopsy of the liver, a diagnosis of syphilitic hepatitis was established. The reduction of liver parenchyma was striking. This patient's T. A. R. was three-plus positive. The other three cases were syphilitic aortitis, aneurysm of the aorta, and tabes dorsalis, respectively.

The last group under the heading of *varia* consists of a number of cases which are of various illnesses, each type having too few in number to be classified individually.

In this group four cases gave a four-plus T. A. R. One was a case of carbon monoxide poisoning, and upon being revived, the patient admitted alcoholism. A second case was that of delirium tremens; a third was given a tentative diagnosis of chronic malaria with a tremendously enlarged liver and spleen; and the fourth case was one of acute cholangitis.

A two-plus T. A. R. was found in a case of impending delirium tremens. In one case of bleeding duodenal ulcer and in another case diagnosed as Banti's disease, the T. A. R. was one-plus positive. Two cases gave a plus-minus reaction, one being a case of acute cholangitis with obstruction of the common duct, and the other a Picks' polyserositis.

The negative T. A. R.'s were found in four cases without objective clinical findings, three cases of common duct obstruction, one compression of the spinal cord, one brain tumor, two retroverted uteri, five cases of chronic cholecystitis, two of arteriosclerotic dementia, three of chronic alcoholism, three of alcoholic coma, one of peptic ulcer, two of malaria, one of acute hydrops of the gall-bladder, one of amygdal poisoning, one of tumor of the right groin (fibroma), one of benign adenoma of the thyroid, one of dysmenorrhea, one of prematurity, one of schizophrenia, and one of acute intestinal obstruction.

In this group, the diagnoses of seven negative T. A. R. cases were confirmed by either postmortem examination or surgery, and one four-plus positive case, diagnosed as a common duct stone, upon laparotomy revealed a stenosis or stricture of the left hepatic duct.

#### DISCUSSION

In summarizing the total number of 276 cases, I find that 114 of those tested gave a positive Takata-Ara reaction varying from a four-plus to a plus-minus positive reaction. The 114 positive reactions were distributed among the different diseases as follows:

Cirrhosis of the liver	50.00%
Acute hepatic insufficiency	4.38%
Arsenical and industrial poisoning	0.87%
Malignant tumors with liver metastases	9.51%
Malignant tumors without liver metastases	2.63%
Normal pregnancies	0.00%
Toxemias of pregnancy	2.63%
Cardiovascular renal disease other than syphilis	1.75%
Diseases of the kidney	0.87%
Acute, subacute, and chronic infections	10.52%
Blood dyscrasias	0.87%
Diseases of the glands of internal secretion	7.01%
Syphilis	0.87%
Varia	7.89%

It is interesting to note that cirrhosis of the liver may be involved in cases of Graves' disease, as has been shown by Rössle,<sup>13</sup> Weller,<sup>14</sup> Assman,<sup>15</sup> and others. Raab and Terplan<sup>16</sup> have reported a case in which subacute yellow atrophy of the liver was a complication of exophthalmic goiter. Weller<sup>14</sup> states that the liver may be, and frequently is, involved in Graves' disease, as shown, clinically, by some occurrences of icterus, marked degrees of which are known to be of serious importance in this disease; physiologically, by the accumulating evidence of altered liver function in such patients; experimentally, by the evidence of hepatic dysfunction following administration of thyroid substance and thyroxin, and morphologically, by structural changes in the liver, varying from slight degrees of chronic hepatitis to a widespread degenerative and necrotizing process which must be considered an acute yellow atrophy.

In Weller's series of 48 cases of Graves' disease, well-marked hepatitis was found in 54 per cent of the cases, while a matched control series of the same size yielded but a single case with a well-marked hepatitis. Youmans and Warfield,<sup>17</sup> in their study, have found that exactly 50 per cent of their cases of hyperthyroidism showed evidence of liver damage.

In reviewing the cases of hyperthyroidism that came to autopsy in the last five years at Cook County Hospital, with the exception of two cases, which were posted and which gave a negative T. A. R. and no marked morphologic changes of the liver, I found that in nineteen cases posted 2, or 10.5 per cent, of the patients had jaundice associated with symptoms of hyperthyroidism, and one patient, or 5.2 per cent, had a peripheral type of cirrhosis of the liver, which is one of the types of cirrhosis found in hyperthyroidism (Rössle<sup>13</sup>). One patient had a slight peripheral fibrous proliferation, and four patients had fatty changes in the liver.

Of the tests running parallel with the positive T. A. R. in this series, in cirrhosis of the liver the ieteric index, as discussed above, showed a parallelism with the T. A. R., but upon comparing the T. A. R. with the ieteric index in the remaining groups, there was no parallelism, although it must be admitted that not all cases had the ieteric index of blood serum run.

The positive Kahn and Wassermann reaction of the serum does not seem to interfere with the Takata-Ara reaction. In a total of 114 positive T. A. R. cases, 14.89 per cent gave a positive Kahn or Wassermann, and 59.89 per cent gave a negative Kahn or Wassermann. Of the negative T. A. R. cases (162), 16.19 per cent gave a positive Kahn or Wassermann and 76.88 per cent gave a negative Kahn.

This finding is in agreement with Tannenholz,<sup>18</sup> who states that the Takata-Ara reactions bear no relationship to the Wassermann or Meinike test. He does state, however, that the T. A. R. is more frequently positive in cases of syphilis (15 per cent) than in other cases (8.5 per cent), such as, genitourinary, dermatologic, and medical cases where a question of liver damage does not present itself.

As to the factors involved in causing the flocculation in a positive Takata and Ara reaction it has been stated by Takata,<sup>1</sup> by Nicole,<sup>12</sup> and Jezler<sup>19</sup> that it is the decreased stability of the serum proteins of the colloid system which makes the precipitation of the colloidal solution of mercuric oxide possible, and that this is due to the increase of globulin with an inversion of the albumin and globulin ratio. It is, according to Nicole,<sup>12</sup> Takata,<sup>1</sup> and others, albumin which exerts a protective action, thus preventing the flocculation of the colloidal solution of mercuric oxide.

In studying the albumin and globulin fractions (after Howe's method<sup>20</sup>), and their ratios, I found that in a total of 23 positive T. A. R. cases, seventeen cases had a ratio of lower than 2:1. If this ratio is taken as the normal, then it would appear to favor the theory of increased globulin content as a factor in causing the flocculation of the mercuric oxysol. However, when I examined 21 cases which gave a negative T. A. R. (Table II), I found that seventeen had a ratio of lower than 2:1.

The globulin in the positive T. A. R. (Table III) does not show an absolute increase, as one would expect to find, according to Jezler's work.<sup>19</sup> In his work on cases with positive T. A. R., the globulin percentage of the total serum proteins was higher than 55, whereas, in the negative T. A. R., the percentage of globulin of the total serum proteins was about 37.

TABLE II  
PROTEIN DETERMINATION IN BLOOD SERUMS WITH NEGATIVE TAKATA-ARA REACTION

DIAGNOSIS	% OF SERUM ALBUMIN	% OF SERUM GLOBULIN	% OF GLOBULIN IN TOTAL ALB. GLOB. CONT.	ALBUMIN GLOBULIN RATIO
Lobar pneumonia	3.88	2.13	33.6	1.82
Lobar pneumonia	4.05	2.00	33.0	2.02
Lobar pneumonia	3.71	2.09	27.7	1.77
Pulmonary tuberculosis	4.36	2.11	32.6	2.06
Pulmonary tuberculosis	3.66	1.96	34.8	1.86
Pulmonary tuberculosis	4.13	2.33	36.0	1.72
T. B. pleurisy with effusion	4.39	2.36	34.8	1.86
Carcinoma of biliary tract	3.87	2.16	35.7	1.79
Carcinoma of head of pancreas	3.90	2.20	34.4	1.77
Carcinoma of stomach	3.71	2.17	31.3	1.74
Carcinoma of liver	4.11	2.19	34.7	1.87
Malignancy with metastases	4.12	2.13	34.3	1.93
Chronic glomerulonephritis	2.88	2.61	47.5	1.10
Lipoid nephrosis	2.40	2.98	55.2	0.80
Hypertensive heart disease	3.71	2.16	36.7	1.71
Hypertensive heart disease	4.00	1.93	32.5	2.07
Arteriosclerotic heart disease	4.44	2.18	32.3	2.03
Pernicious anemia	3.18	1.97	38.2	1.67
Exophthalmic goiter	3.33	2.00	37.5	1.66
Toxic hepatitis	3.33	1.94	36.8	1.71
Cirrhosis of liver	3.73	2.46	39.7	1.51

Schindel,<sup>21</sup> by adding various concentrations of lower fatty acids such as formic, acetic, or propionic acids, to a dilution series of a serum, was able to produce a positive T. A. R. in a serum which otherwise gave a negative T. A. R. The addition of mineral acids, however, did not have this effect. The flocculations, according to Schindel, took place exactly as in the naturally positive T. A. R. serum. He was also able to transform the experimental as well as the naturally positive T. A. R., by the addition of sodium or potassium hydroxide, into a negative T. A. R. Nicole,<sup>12</sup> on the other hand, in his experiments with various concentrations of albumin and globulin derived from horse serum, was able to show that albumin is a factor in producing the various changes in color, whereas globulin is a factor in causing flocculation. When these proteins were mixed in normal proportions, the T. A. R. was negative, whereas, if the globulins were increased or the albumins decreased, a positive T. A. R. occurred.

Another interesting observation was made by Zirm,<sup>22</sup> who, with the addition of heparin to the blood serum, was able to prevent in a positive serum the occurrence of a flocculation, whereas a previously negative serum did not manifest any change. In his series 5 mg. of heparin per tube in one set and 20 mg. per tube in another set were used, and those tubes with the greater amount of heparin showed less flocculation than those tubes with the smaller amount of heparin.

I have carried out similar experiments, and the results are very striking, in that in some of the cases the use of as little as 1 mg. of heparin per tube



TABLE III

PROTEIN DETERMINATION IN BLOOD SERUMS WITH POSITIVE TAKATA-ARA REACTION

DIAGNOSIS	% OF SERUM ALBUMIN	% OF SERUM GLOBULIN	% OF GLOBULIN IN TOTAL ALB. GLOB. CONT.	ALBUMIN GLOBULIN RATIO
Cirrhosis of liver	4.0	1.96	32.8	2.04
Cirrhosis of liver	3.87	2.09	35.0	1.85
Cirrhosis of liver	3.91	2.10	34.9	1.86
Cirrhosis of liver	3.66	1.93	34.5	1.89
Cirrhosis of liver	3.97	2.21	35.7	1.79
Cirrhosis of liver	3.77	1.92	33.7	1.96
Cirrhosis of liver	4.26	2.08	32.8	2.04
Cirrhosis of liver	4.08	2.11	32.6	1.93
Cirrhosis of liver	4.63	2.37	33.8	1.53
Cirrhosis of liver	3.90	2.12	35.2	1.83
Cirrhosis of liver	4.11	1.95	32.1	2.10
Cirrhosis of liver	4.19	2.14	33.8	1.95
Cirrhosis of liver	4.05	1.96	32.6	2.06
Cirrhosis of liver	3.74	2.09	35.8	1.78
Syphilitic cirrhosis of liver	4.00	2.31	36.4	1.75
Acute hepatitis	4.37	2.41	35.5	1.81
Exophthalmic goiter	3.71	2.12	36.3	1.75
Exophthalmic goiter	3.84	2.31	39.1	1.66
Exophthalmic goiter	3.94	2.32	37.0	1.69
Exophthalmic goiter	3.91	1.96	34.0	1.99
Carcinoma of pylorus of stomach with metastases	3.93	2.14	35.2	1.83
Chronic malaria with severe anemia	4.50	1.97	30.6	2.28

prevents the flocculation of mercuric oxide. The following chart shows some of the results obtained.

	Flocculation in tube							
	1	2	3	4	5	6	7	8
Cirrhosis of the liver without heparin	0	0	+1	+1	+4	+4	+3	+2
Cirrhosis of the liver with heparin, 3 mg. per tube	0	0	0	0	0	0	0	0
Cirrhosis of the liver without heparin	0	0	+3	+4	+4	+4	+3	+2
Cirrhosis of the liver with heparin, 1 mg. per tube	0	0	0	±	±	0	0	0

It is obvious from the above observations and the differences of opinion in the literature that the chemistry involved is perhaps more complicated than it seems, and it requires much further investigation to determine the actual factors concerned in the cause of flocculation in the positive T. A. R.

## SUMMARY

1. Two hundred and seventy-six cases with the Takata-Ara reaction have been studied.
2. That this is of value in cases of decompensated liver conditions due to cirrhosis is without doubt. For latent liver damage the test is not entirely reliable.
3. The T. A. R. is positive in 98 per cent of the cases of cirrhosis of the liver.
4. The simplicity with which the test is carried out warrants its use in hospital and office practice as both a diagnostic and prognostic aid in cases of cirrhosis of the liver.

5. Liver impairment occurs in hyperthyroidism, as shown by the Takata-Ara reaction.

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# ARTIFICIAL PNEUMOTHORAX IN THE TREATMENT OF LOBAR PNEUMONIA\*

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A REVIEW OF FORTY PNEUMOTHORAX-TREATED PATIENTS AND ONE HUNDRED  
PATIENTS TREATED BY OTHER METHODS

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ALBERT BEHREND, M.D., PHILADELPHIA, PA., VERNON L. TUCK, M.D., SHERMAN,  
TEXAS, AND WILLIAM EGBERT ROBERTSON, M.D., PHILADELPHIA, PA.

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IN A RECENTLY published article one<sup>1</sup> of us reported the results obtained in the treatment of eleven cases of lobar pneumonia by artificial pneumothorax. It was emphasized at that time that, although the procedure gave promise of being a worthy addition to the armamentarium in the treatment of lobar pneumonia, the number of cases treated was too small to enable us definitely to evaluate it. We have now had the opportunity to observe carefully forty patients treated by artificial pneumothorax.

Inasmuch as any study of this nature is of little value without the benefit of a control group of cases taken at the same time of the year, and under as nearly similar conditions as possible, 100 cases of lobar pneumonia treated by methods other than pneumothorax are also presented.

## THE THEORY OF THE ACTION OF ARTIFICIAL PNEUMOTHORAX IN PNEUMONIA

Lilienthal<sup>2</sup> has suggested that since care is taken not to produce a positive intrathoracic pressure, partial collapse rather than compression occurs when air is introduced. Partial collapse is probably the most accurate way of describing the state of a pneumonic lung treated by artificial pneumothorax. In any case, a lung partially or completely surrounded by a layer of air is splinted to a lesser or a greater degree. This has been shown roentgenographically and at post-mortem. The acutely inflammatory tissues of the diseased lung acquire at least a relative degree of rest because complete expansion is prevented. The parietal and visceral pleurae are separated and the pain of attendant pleuritis is abolished almost without exception. These facts we know because they have been proved clinically again and again.

The ability of air to produce even a partial degree of collapse in a pneumonic lung that is in the stage of hepatization has frequently been questioned. Clinical, roentgenographic, and postmortem findings lead us to the belief that partial collapse of such a lung can occur. The term "hepatization" brings to mind the tense and sharply defined lung as it lies on the autopsy table. We question whether this mirrors the true condition of such a lung during life. In death

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\*From the Medical and Tuberculosis Services of the Philadelphia General Hospital.  
Received for publication, October 3, 1934.

the cellular and fluid exudates rich in protein content are coagulated. Such a lung would indeed render collapse impossible. That body fluids high in protein content frequently gel when removed from the body is common knowledge. Similarly the hepatized lung may be regarded as a sac containing a large amount of fluid rich in protein and a relatively small amount of air. That such an organ can be partially collapsed is possible. That it can be collapsed sufficiently to prevent further pleuritic pain, we know.

How does pneumothorax bring about crisis in lobar pneumonia? That it does so in a certain number of cases we feel relatively sure. Here is ample field for theoretical speculation and laboratory investigation. It has been shown that artificial pneumothorax causes a decrease in the amount of blood circulating in the lung so treated. Lymph stasis with diminution in the absorption of toxins has also been shown to occur after pneumothorax. Whether or not crisis is the product of the sum of these factors acting in conjunction with the natural immunologic resources of the organism can only be surmised.

More or less subjective improvement promptly followed artificial pneumothorax in the majority of cases of lobar pneumonia so treated, irrespective of the ultimate outcome. The reaction in some is clinically similar to that seen after the intravenous injection of a foreign protein, except for the absence of the initial chill. The critical fall in temperature and the objective and subjective improvement in the patient lead one to speculate on the cause. One of us (W. E. R.) has been inclined to regard it as a protein reaction induced by the air, which very probably forces altered proteins into the blood stream from the involved lung. Differential blood counts, however, do not entirely support this view, for the well-known phase reactions following intravenous protein injections, are not so pronounced a feature after pneumothorax.

#### SELECTION OF CASES FOR ARTIFICIAL PNEUMOTHORAX

All the patients treated in this series were believed to have unilateral lobar pneumonia involving one or more lobes at the time pneumothorax was induced. As soon as the diagnosis was established by physical signs or confirmed by roentgenographs, air was introduced. In an effort to determine the true value of the treatment, all patients admitted to our services that fulfilled the requirement of unilateral lobar pneumonia were given air. Some of the patients appeared to be doomed at the time treatment was begun. Occasionally this type of patient surprised us by recovering, but usually the treatment was of no avail in preventing death. Seven patients were women, thirty-three were men.

Patients were given air regardless of the day of disease. This varied from the first to the fourteenth day, although the first pneumothorax in the majority of cases was given on the third, fourth, or fifth day. Many of the most brilliant results occurred in this group. It seems to us that those who use artificial pneumothorax only on or before the third day, as has been advocated, limit its application needlessly and deny the patient therapeutic aid at a time when it is best utilized and when the response of the patient is most gratifying.

The amount of air given was usually between 400 c.c., and 600 c.c. and in most cases was repeated in twenty-four hours. This amount was used because

it is sufficient to accomplish the desired result without causing discomfort to the patient or a mediastinal shift. At no time was a positive intrapleural pressure produced, the needle being always withdrawn before this stage was reached. Occasionally it was impossible to introduce more than 200 c.c. of air because of pleuritic adhesions. In only one case did adhesions altogether prevent the establishment of pneumothorax.

#### SELECTION OF CASES FOR CONTROL GROUP

The first one hundred patients admitted to the wards with lobar pneumonias after Dec. 1, 1933, and not treated by pneumothorax, comprise the control group. Of these, eighty-four were men, sixteen were women. Since there are ten medical services at the Philadelphia General Hospital, and artificial pneumothorax was used on only one or two, controls were abundant. Specific serums could only be given when the patient or his friends could afford to buy it, so that it was used in only two cases. One of these had a nonspecific immunotransfusion in addition. Another patient received nonspecific protein therapy in the form of 50,000,000 killed typhoid organism intravenously. The remainder of the patients were treated by the usual supportive measures and those designed to give symptomatic relief.

#### RESULTS OF TREATMENT

The results of treatment are presented in compact form in Table I. A few facts are worthy of special mention.

TABLE I  
SUMMARY OF DATA

AGE, YEAR RANGE	SEX		COL-OR		MODE OF FALL OF TEMPERATURE		ORGANISM														DEFERESCENCE, MEAN, DAYS	ARTIFICIAL PNEUMO-THORAX		DIED	RECOVERED
							SPUTUM							BLOOD											
	M	F	W	B	LYSIS	CRISIS	PNEUMO-COCCUS				B. FRIEDLANDER	PNEUMO-COCCUS				B. FRIEDLANDER	UNKNOWN	NO GROWTH	DAY AFTER ONSET OF FIRST INJECTION, MEAN	AIR INJECTED, RANGE C.C.					
							TYPE 1	TYPE 2	TYPE 3	GROUP 4		TYPE 1	TYPE 2	TYPE 3	GROUP 4										

Cases 1 to 40 inclusive, treated by artificial pneumothorax																							
13-69	33	7	18	22	16	18	3	4	0	13	1	2	1	0	8	0	0	11	3.42	4.2	50-600	14	26

Cases 41 to 140 inclusive, treated by other methods																								
12-75	84	16	63	37	38	9	1	5	0	12	0	4	11	2	5	1	1	15	8.57				51	49

*Relief of Pain.*—That artificial pneumothorax relieves the pain so frequently associated with respiration in lobar pneumonia all observers agree. Relief is experienced almost immediately after the needle is withdrawn and is promptly noticed by the patient. The need for narcotics is in consequence greatly diminished. Relief of pain is one of the most spectacular results of the use of artificial pneumothorax in the treatment of lobar pneumonia. One of us (V. L.

T.), who treated twenty-five of the forty cases in this series, has noted that patients frequently requested refills of air after rapid absorption had allowed a partial return of pleuritic pain.

*Relief of Cyanosis, Dyspnea, and Toxicity.*—Cyanosis is less frequently encountered than pain in early pneumonias. It is our experience that when present it is relieved by pneumothorax. Dyspnea is frequently associated with pain. When pain is relieved by the introduction of air, dyspnea usually disappears. "Toxicity" is a rather abstract term, and has to be judged by the appearance and the mental state of the patient. In one patient jaundice was present on admission but disappeared within twenty-four hours. When a toxic psychosis is well established, pneumothorax will not abort it, but we have seen patients apparently on the threshold of delirium benefit remarkably and take a renewed interest in themselves and their surroundings. It is certain that, in general, patients look less sick after pneumothorax has been induced, but when a toxic psychosis does occur, it makes the prognosis decidedly more grave.

#### MORTALITY

In the control group of one hundred cases, there were fifty-one deaths, a mortality of 51 per cent. This is an appallingly high death rate, and yet it is substantially in accord with figures which are reported from other large general hospitals. Here is evidence enough that there is need now, as there has been for the past two hundred years, for an effective agent in the treatment of lobar pneumonia, an agent that must be inexpensive, yet potent without regard to the type of organism causing the infection.

In the pneumothorax-treated group there were fourteen deaths, a mortality of 35 per cent. Three of these died within eight hours after admission to the hospital, and were almost moribund when treated. That we have been able to reduce mortality by 16 per cent in the worse types of pneumonia suggests the hope that those who treat more favorable cases can do even better.

One of the deaths occurred in a patient with a toxic psychosis whose temperature fell from 104° to 96° twelve hours after the second injection of air. The psychosis persisted despite the febrile drop and the patient inadvertently got out of bed that day. He died a few hours after he was put back to bed.

Another death occurred in a patient who had been afebrile for five days. During all this time he had a low muttering delirium from which it was impossible to arouse him, and death was apparently due to myocardial exhaustion. There was no postmortem examination.

Two cases showed acute vegetative endocarditis at autopsy.

#### PERIOD OF DEFERVESCENCE

Table I shows the mean duration of fever during the hospitalization of patients who recovered. After the first pneumothorax the average duration of fever was three and a half days. In the control group the average duration of fever was seven days. It appears, then, that pneumothorax shortens the febrile course. This in turn means an abbreviated convalescence and conservation of

the energy reserves of the patient. In addition, the economic benefit which the patient and those who pay for his hospitalization derive from his shortened stay is notable.

#### PRODUCTION OF CRISIS

In eighteen of the forty patients treated by induced pneumothorax, the temperature fell by crisis, using the term to denote a fall in temperature to normal within twenty-four hours after the first injection of air. The temperature does not then remain normal in all cases, and this is the main reason for repeating the treatment within twenty-four hours. Two injections usually suffice, but there should be no hesitation in giving others if they seem indicated. The fact that crisis is induced does not of necessity imply that the patient will recover. Six of the eighteen patients died despite that fact, the temperature usually rising again after a preliminary drop, and remaining elevated despite the addition of more air.

In sixteen cases temperature fall by lysis was observed. It was impossible to predict whether temperature would fall by crisis or by lysis.

In six cases pneumothorax produced no temperature change and in one of these only 150 c.c. of air could be introduced, because of the presence of adhesions.

Among the one hundred patients treated by methods other than pneumothorax, spontaneous crisis occurred only seven times. These patients all recovered.

#### COMPLICATIONS

In the pneumothorax-treated group:		In the control group:	
Delayed resolution	1	Thrombophlebitis	1 (recovered)
Pericarditis	2 (both recovered)	Suppurative otitis media	1 (died)
Acute vegetative endocarditis	1 (died)	Jaundice	1 (died)
Empyema	1 (recovered)	Pneumococcal meningitis	1 (died)
Pneumococcal meningitis and acute vegetative endocarditis	1 (died)	Pleural effusion	3
Jaundice	1 (recovered)	Acute arthritis	1
		Empyema	3 (all recovered)
		Lung abscess	2 (1 died, 1 recovered)
		Delayed resolution	3
		Acute vegetative endocarditis	2 (died)

There is apparently little difference in the occurrence of complications in the two series (17 per cent in pneumothorax-treated cases, 18 per cent in those treated by other methods).

#### RESULTS OF BLOOD CULTURE

In patients with an established pneumococemia artificial pneumothorax was of little benefit. Five of the 40 patients treated returned positive blood cultures, and of these only one recovered.

In the control group of 100 cases there were 27 cases of bacteremia and of these patients 6 recovered and 21 died.

The results in this group are rather striking, and may or may not be significant. According to our figures, the number of cases of bacteremia occurring in the cases treated by artificial pneumothorax was less than half (12 per cent) that of the number that appeared in the control group (27 per cent).

#### SUMMARY

A series of 40 patients with lobar pneumonia treated by artificial pneumothorax is presented, with a mortality of 35 per cent. A control group of 100 patients with lobar pneumonia treated by other methods is also presented, with a mortality of 51 per cent. In addition to a decreased mortality, the chief benefits of artificial pneumothorax are relief of pain, dyspnea, cyanosis, and "toxicity." It also shortens the febrile period sometimes by crisis, sometimes by lysis, and so lessens the number of hospital days per patient. Artificial pneumothorax is of little aid in the treatment of well-established pneumococemias; it will not abort toxic psychoses, and it apparently has little influence on the prevention of the more severe complications of lobar pneumonia.

#### CONCLUSION

One must conclude from the results of this study that artificial pneumothorax offers greater hope for recovery, greater comfort during illness, and a shorter period of convalescence than the ordinary nonspecific measures which strive for symptomatic relief in patients suffering from unilateral lobar pneumonia.

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## PHOSPHORUS METABOLISM

### V. RELATION BETWEEN URINARY PHOSPHATE AND BLOOD PHOSPHOLIPIDS DURING ABSORPTION OF FATS

GUY E. YOUNGBURG, PH.G., PH.D., BUFFALO, N. Y.

IT HAS been shown by a number of investigators that the phospholipids of the blood of animals increase on a high-fat meal. In dogs Bloor<sup>1</sup> found an increase in whole blood of as high as 70 per cent and never less than 30 per cent, when 50 to 100 c.c. of olive oil alone was fed. Recently<sup>2</sup> he found that in single overfeedings of dogs with fat or carbohydrate the increase in plasma phospholipids averaged 28 per cent. There was no increase with protein. So far as the phospholipids of the human blood are concerned it is known that the content, under similar conditions of food, environment, etc., is about the same as in dogs and many other animals.

Assuming that the whole blood increases about 33 per cent in phospholipid within about four to six hours after a meal (from a content of 0.3 up to 0.4 per cent), in other words an increase of 0.1 per cent, we can calculate that the blood of an average person would then contain 4.5 gm. more phospholipid than previously (0.001 by 4,500 c.c.). Since the phospholipids contain about 4 per cent of phosphorus, the increase represents 180 mg. of that element. This is not an inconsiderable quantity, and if it is derived directly from the same phosphorus compound in the body as the urinary phosphate, we might expect the latter to be decreased. The source for urinary phosphates is in all probability the inorganic phosphate of the blood,<sup>3</sup> although evidence has also been presented especially by Eicholtz, Robison and Brull<sup>4</sup> which makes it somewhat inconclusive. Whatever the facts are in this regard, if the blood phospholipid increase represents newly-formed phospholipids, we would expect that the P from both they and the urinary phosphates would be derived from the same immediate and potential source. Thus, an increase in blood phospholipids would result in a decrease in urinary phosphates.

The purpose of this investigation was to determine if the blood phospholipid increase in reality does decrease the urinary phosphate.

#### EXPERIMENTAL

Four normal men twenty-one to twenty-five years of age were selected as subjects. Their urines were carefully collected in one-hour periods for eight consecutive hours, beginning at 7 A.M., with the usual breakfast of each subject soon after 8 A.M. The usual amount of water was allowed but no other food than the breakfast. The volumes and the phosphorus contents of the urines were determined. The results are shown in Table I. The table also shows the results

\*From the Department of Biological Chemistry, University of Buffalo Medical School.  
Received for publication, October 5, 1934.

TABLE I\*  
 INORGANIC PHOSPHORUS IN URINE OF MEN, ON USUAL AND ON HIGH-FAT BREAKFASTS (MG. P PER HOUR)

TIME	SUBJECT M			SUBJECT C			SUBJECT A			SUBJECT H			TOTAL	
	USUAL BREAKFAST	HIGH-FAT BREAKFAST		USUAL BREAKFAST	HIGH-FAT BREAKFAST		USUAL BREAKFAST	HIGH-FAT BREAKFAST		USUAL BREAKFAST	HIGH-FAT BREAKFAST		USUAL BREAKFAST	HIGH-FAT BREAKFAST
7-8 A.M. breakfast	46.2	77.2		51.8	98.0		39.1	27.3		44.4	40.6		181.5	243.1
8-9	19.3	20.1		20.8	29.1		28.4	32.2		48.6	36.1		117.0	117.5
9-10	6.2	7.4		20.8	51.6		19.9	30.5		24.1	32.2		71.0	121.7
10-11	21.1	5.5		14.6	30.8		28.0	28.9		22.5	28.9		86.2	103.1
11-12	30.8	15.8		18.7	41.1		25.8	51.6		27.6	23.7		102.9	132.2
12-1 P.M.	36.5	36.2		28.1	11.4		37.3	32.4		26.2	24.5		128.2	104.5
1-2	40.3	8.7		24.5	19.5		45.0	38.0		19.8	44.5		129.7	110.7
2-3	31.9	37.1		21.7	41.5		44.0	38.6		26.7	45.9		124.4	163.1
Total	232.4	208.0		200.9	332.0		267.6	279.5		240.0	276.4		940.9	1095.9

\*Body weight: Subject M, 70.4 kg.; C, 64.5 kg.; A, 71.8 kg.; and H, 88.2 kg.

of a similar run with the same subjects a week later, except that during the breakfast 75 gm. of corn oil (Mazola) was ingested by each. This amount of oil is about the maximum that can be taken by the average person without causing considerable nausea or laxation.

### RESULTS AND DISCUSSION

The tabulated results show that the formation of urinary phosphate is not influenced or diverted to form phospholipid in the body when a high-fat meal is taken; there is even somewhat more phosphate excreted under the fat meal. It can be observed on closer inspection of the table that there are decreases in phosphorus outputs during some parts of the eight-hour periods under the fat meals, but the decreases are irregular and cannot be ascribed to phospholipid formation from absorbed fat. Therefore it cannot be concluded that the phosphorus of blood phospholipid and urinary phosphate are drawn from the same immediate source.

There is no correlation between excretion and urine volume. This is already well known and the figures for the urine volumes are therefore not included.

Most investigators have accepted the phospholipid rise in the blood during fat absorption as a neogenesis for purposes of transportation and intermediary metabolism of the fatty acids. This has been the starting point for extensive consideration of the phospholipids in this regard. Recently Sinclair,<sup>5</sup> on the basis of his experiments, has suggested the hypothesis that the absorbed fatty acids are transformed into phospholipid within the intestinal mucosa as an essential step in the resynthesis of neutral fat. His data indicate that there is, however, no increase in phospholipid content in the intestinal mucosa when fats are absorbed. It seems possible that neogenesis in the wall during fat absorption results in phospholipid flow into the blood and that this accounts for the nonrise in the mucosa. The phospholipids could thus function both in fat absorption and fat transportation. (Other possible functions of phospholipids do not appear to be connected with this research.)

The results of this research indicate the probability that the increase in blood phospholipids represents such lipids mobilized from some other part of the body, whether it be from the intestinal wall, the liver, or the spleen, for example, and that this increase is not closely connected with the excretion of phosphates in the urine.

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## COMPLEMENT FIXATION REACTIONS IN CARCINOMA\*

HOWARD W. LUNDY, M.S., ST. LOUIS, MO.

A CONSIDERABLE number of investigators have studied the alcohol soluble heterophile antigen extractable from carcinomatous tissue and have concluded that there exists a cancer specific antigen. In large part, these conclusions have been reached as a result of experiments in which the antigen, more or less purified by solution or precipitation with various organic solvents, has been injected into rabbits and a specific antibody had been shown to develop. In addition, efforts have been made to demonstrate by means of the complement fixation test the presence of antibodies in the blood of cancerous patients which react specifically with the heterophile carcinoma antigen.

Hirszfeld and Halber<sup>1</sup> announced in 1930 that in 10 or 20 per cent of rabbits which were injected with alcoholic extracts of cancer tissues, antibodies were obtained which reacted with 60 per cent of all tumors tested while these antibodies did not react with normal tissues. They also tested 300 cancer and 1,700 normal serums with cholesterinized alcoholic extracts of cancer tissue and found 50. to 60 per cent of these serums positive in cases of cancer of the inner organs but only rarely in cases of cancer of the external tissues. If the serums were Wassermann positive, they were also positive with the cancer antigen.

Witebsky and Pöplau<sup>2</sup> showed that cancer tissue might be boiled for thirty minutes without losing the ability to produce specific antibodies when injected into rabbits. They found that subsequent to the injection of cancer material in animals, the development of cancer specific serum alterations depend mainly on the particular cancer material used but also on the individual rabbit.

Cohn and Collier<sup>3</sup> found no antibodies resulting from the injection of rabbits with the plain alcoholic extract but when they mixed the extract with pig serum before injecting it specific antibodies were temporarily produced. Sievers<sup>4</sup> found reactions occurring with both immune and normal rabbit serums when using cancer extracts. He believes that the complement fixation results from a physical-chemical change. Recently, Saphir and Hirschberg,<sup>5</sup> using cholesterinized alcoholic extracts, found positive fixations in 77.7 per cent of 27 cases of cancer. Only one case out of 82 normal controls was positive. All of the serums which they tested were Wassermann negative. These workers also think that the reaction is of a physical-chemical nature due to the action of the antigen on the serum globulin.

The older tests were negative for specificity because the crude alcoholic extract which was used as antigen contained mixtures of various substances. These substances react with a variety of antibodies and it is only by their removal that specific reactions may hope to be obtained.

\*From the Department of Bacteriology and Hygiene, St. Louis University School of Medicine.

Received for publication, October 6, 1934.

A distinct advance was made by Lehmann-Facijs.<sup>6</sup> He realized the necessity of purifying the antigen and so fractionated the alcoholic extract by use of certain organic solvents. That fraction which was soluble in ether, benzene and alcohol but insoluble in acetone he found to contain the cancer antigen. This fraction he thought to be a phosphatid. By means of this fractionation he got rid of certain fatty acids, fats and cholesterol esters and greatly increased the reactivity of the antigen. Like previous workers he found that syphilitic serums likewise gave positive reactions with the extract.

Morelli,<sup>7</sup> using a limited number of extracts, reported that she could not separate the various fractions by this method of Lehmann-Facijs.

In the present work two points have been studied: first, does the heterophile syphilitic antigen give specific positive reactions with the bloods of cancerous patients? Second, is it possible to separate the syphilitic antigen present in the cancer tissue extract from the specific cancer antigen?

The method of preparation of the antigen used in these experiments was essentially the same as that recommended by Lehmann-Facijs. A fresh specimen of cancer tissue was obtained from the operating room or autopsy room and all normal tissue and fat removed with the aid of scissors. The tissue was then ground in a food grinder and placed in a flask containing glass beads together with five times the volume of 95 per cent alcohol. The tissue was then extracted at 37° C. for eight days, shaking the flask several times a day.

At the end of this time the extract was filtered through a double filter paper and the filtrate constituted the alcoholic extract. For purposes of fractionation 40 c.c. were placed in a large test tube and evaporated to dryness in a water-bath. The residue was dissolved in 40 c.c. of benzene, the solution filtered and the filtrate again evaporated. The residue was dissolved in acetone and the insoluble fraction collected by centrifuging the mixture. This insoluble portion was then dissolved in petrol ether, filtered and again evaporated. The residue was dissolved in 95 per cent alcohol, the solution filtered and the final volume adjusted to one-half of the original. This fraction corresponds to the phosphatid fraction of Lehmann-Facijs.

In the later antigens which were prepared, ether and chloroform were used in the extraction in addition to the other solvents as a further purification of the antigen was apparently obtained when these solvents were employed. The active material was soluble in these reagents. These solvents were used after the petrol ether.

Ethylene chloride was also employed as a solvent. The value of this reagent was discovered while conducting a series of experiments with solvents, having higher boiling points, on the destruction of the antigen by heat. It apparently gives quite promising results. The acetone insoluble fraction was dissolved in one-half the volume of ethylene chloride, the solution filtered and the filtrate evaporated as before. The residue was dissolved in one-fourth the original volume of 95 per cent alcohol and the resulting antigen was quite active even without cholesterinization.

By extracting each antigen several times either with each reagent or with certain reagents a further purification might be accomplished. This procedure was employed in the more recent extractions and it was found to be of value.

When the antigen was kept for periods longer than a month it became increasingly anticomplementary and in spite of dilution it was no longer usable.

Cholesterinization of some of the antigens was carried out and was performed by using the technic of Hirszfeld and Halber.<sup>8</sup> To 0.35 c.c. of the extract was added 0.15 c.c. of a 1 per cent alcoholic solution of cholesterin. Three cubic centimeters of saline were then quickly added and the material mixed with shaking. In this form the antigen was very unstable, and it had to be prepared just before it was used.

A Wassermann antigen was also prepared according to the method recommended by Kolmer and the cancer serums were all tested for their reaction with the syphilitic antigen.

The patient's blood was inactivated at 56° C. for twenty minutes before using it. This inactivation was carried out very carefully as heating a positive serum at 58° for fifteen minutes seemed to destroy the antibodies reacting with the cancer antigen. In all the experiments increasing dilutions of the serum from cancer patients were used in testing the cancer antigens. A 2 per cent suspension of sheep cells was used in the indicator system. Two units of amboceptor contained in 0.5 c.c. were used in the set-up.

The antigen was titrated for its hemolytic, anticomplementary and antigenic power. It was used in a dilution which was ten times the titrated strength, thus ten units were used; this value was always removed by at least ten or more units from the hemolytic and anticomplementary titers or the antigen was considered unfit for use.

For the test proper nine tubes were set up for each serum to be examined. The first four contained serum dilutions of 1-5, 1-10, 1-20, and 1-40, respectively. The fifth tube contained a 1-5 dilution of the serum but saline was added in place of the antigen. The sixth tube contained four-plus syphilitic serum diluted 1 in 5 and the seventh the same serum but saline in place of the antigen. The eighth and ninth tubes contained normal serum diluted 1 in 5, with saline replacing the antigen in the ninth tube. The complement was titrated each day just before using it. Readings of the final results were noted as four-plus, three-plus, etc., depending on the degree of inhibition of hemolysis.

Twenty-one antigens were made according to the Lehmann-Facijs technic. Not all of them were suitable for use, however, as some did not exhibit sufficient difference between the fixing unit and the anticomplementary unit to meet the requirements specified above. As a rule, the hard, firm tissue made the best antigen; the softer necrotic tissue was less serviceable. Cancer of the inner organs provided better antigens than did growths from the outer tissues. This is in agreement with the results obtained by Marzynsky and Silberstrom<sup>9</sup> and opposite from those of Hirszfeld and Halber.

Of seven tumors of the breast only two furnished satisfactory antigens; of six tumors of the skin only one furnished a satisfactory antigen; of three tumors of the gastrointestinal tract (stomach, colon and rectum) all gave usable ex-

TABLE I

COMPLEMENT FIXATION BY SERUM FROM CANCER CASES USING AS ANTIGEN "PHOSPHATID"  
ANTIGEN FROM CANCERS

LOCATION OF TUMOR	TOTAL	TOTAL NUMBER POSITIVE	NUMBER POSITIVE AND EARLY CASE	TOTAL NUMBER NEGATIVE	NUMBER NEGATIVE AND EARLY CASE
Breast	12	12	—	—	—
Breast	1	—	—	1	—
Metastasis from body of uterus	4	4	—	—	—
Cervix uterus	5	5	—	—	—
Rectum	6	5	—	1	—
Stomach	1	—	—	1	1
Skin (various locations)	8	2	1	6	5
Buccal cavity	2	2	2	—	—
Tongue	2	—	—	2	2
Larynx	1	1	—	—	—
Esophagus	2	1	1	1	1
Unknown	7	4	—	3	1
Totals	51	36	4	15	10

tracts; three metastatic tumors (lung, liver and pelvis) also gave good antigens; and one tumor of the testicle gave a good antigen.

The antigenic titer of the acetone insoluble fractions of the antigen varied between 1.150 and 1.1500 for cancer serum and between 1.50 and 1.100 for syphilitic serum. The selected cholesterinized antigens fixed the complement in titers of 1.400 to 1.4000 in the presence of cancer serum and between 1.300 and 1.2000 with syphilitic serum. The ethylene chloride fraction gave a titer of 1.4000 to 1.5000 with the cancer serum and less than 1.1000 with syphilitic serum. It is apparent that quantitatively there does seem to be a certain limited degree of specificity of the cancer antigen. This was, however, not evident in all the cancer antigens. It also appears that the ethylene chloride fraction shows this specificity more markedly than the other less highly purified antigens.

The serums from fifty-one cases of carcinoma were tested with Lehmann-Facius' phosphated antigen, either with or without addition of cholesterol and positive reactions obtained in about 70 per cent (Table I). All of these serums were Wassermann negative. One hundred and seven syphilitic serums which gave a 4+ reaction with the Kolmer antigen were examined with the same antigens and all but two of them reacted positively with the acetone insoluble fractions prepared according to Lehmann-Facius. With ninety-two normal control serums one positive reaction was obtained. Ten serums from tuberculous patients gave negative reactions as did two from cases of adenoma of the breast.

These results compare quite favorably with those of Simon and Thomas<sup>19</sup> and Hirschfeld and Halber. As has been stated, it is generally accepted that the alcoholic extracts from cancer tissue will react with syphilitic serums and even upon fractionation of the antigen not all of the syphilitic antigen is removed. Our results also bear out the claims of Lehmann-Facius that a more active, purified form of the antigen may be obtained by the method of fractionation used.

The patient's serum gave stronger reactions when the involvement by the growth was more extensive. There was quite a close correlation between the extent to which the cancer had advanced and the reaction obtained. Practically

all of those serums giving negative reactions were from patients upon whom the diagnosis of cancer in the early stages had been made.

The use of ethylene chloride in the fractionation allowed an earlier detection of cancer by this reaction than did the plain phosphatid fraction. Serums that were negative with the latter antigen gave two- and three-plus reactions with the ethylene chloride antigen. Only three satisfactory antigens were prepared by this method and twenty-eight serums tested so that the results are not as valuable as if a greater number had been used. All twenty-eight serums were, however, positive. Several of these serums were from cases of early carcinoma and some were from cases which had given a negative reaction with the Lehmann-Facijs phosphatid antigen.

Twenty-seven syphilitic serums were tested with these three ethylene chloride antigens and sixteen were positive while eleven were negative. This apparently indicates a purification of the cancer antigen and removal of some of the Wassermann antigen.

On the other hand, out of eleven normal serums tested with this purified antigen, four gave positive reactions. This would seem to indicate that the purification of the antigen had made it too reactive.

The fact that syphilitic serums gave reactions with extracts from cancer tissue was recognized quite early by the men in this field. Hirszfeld and Halber, Witebsky and Pöplau, Marzinsky and Silberstrom, Lehmann-Facijs and others all finding fixation with syphilitic serums. This may be explained either on the basis that the cancer antigen contains the syphilitic antigen also or that the syphilitic serum contains the cancer antibodies. Lehmann-Facijs believes the former to be true and this is borne out in the present work.

On the other hand, Hirszfeld and Halber<sup>11</sup> think that it is the antigen found in the blood Group A or AB individuals that is active. In order to check this concept antigens were prepared by alcohol extraction of erythrocytes of several Group A individuals and also one Group B individual. None of the antigens so prepared gave positive complement fixation with either serum from syphilitic or cancerous individuals. In the present work all but six of the bloods from cancer patients were typed, but as far as could be determined the grouping did not influence the results.

In typing the bloods of the cancer patients in regard to the blood groups it was noticed that there was an unusually high number of individuals falling in Group A. This fact does not seem to be related to any known factor. Of the 45 persons typed who were suffering from a cancer, 55 per cent were in this group.

A further indication that both syphilitic and cancer antigens are present in the cancer extract is the fact that the cancer antigen is destroyed by certain alkaline reagents while the syphilitic antigen remains intact. This was accomplished by dissolving the acetone insoluble residue of the cancer extract in pyridine, filtering, dissolving the residue obtained after evaporating the pyridine in amyl alcohol, filtering of the insoluble portion and evaporating off the amyl alcohol. When this residue was dissolved in 95 per cent alcohol and tested for its antigenic power with cancer positive serum, the fixing ability had entirely



disappeared. The same is true when using ethylene diamine, and yet fixations were obtained with these same fractions with syphilitic serums. When ethylene glycoll is used, both the antigens are destroyed.

The destruction of the cancer antigen was shown not to be due merely to the heat necessary to evaporate the solvent. By using distillation in the vacuum chamber the liquids boiled under 70° C. and still the cancer antigen was destroyed. By heating the acetone insoluble fraction in a cotton seed oil bath, it was found to be stable at 125° C. for half an hour and gave fixation with cancer and syphilitic serum. From these results it would seem that both the cancer antigen and the syphilitic antigen are present in the cancer extract and that they have characteristically distinct properties.

If this assumption is made, the problem still remains to separate the two substances and the most promising method for accomplishing this seems to be fractionation and repeated extraction with various organic solvents. There is the danger, of course, that this may be carried too far and one may obtain a nonspecifically sensitive antigen. The ethylene chloride fraction gave several positive reactions with normal serums, and this is a factor which must be taken into consideration. With the phosphatid fraction only one normal control serum gave a positive test.

It seems evident from these studies that the antigens which are active in syphilitic complement fixation reactions and in cancer fixation reactions are probably not identical, and it should be possible by further study to separate these two antigens. It does not necessarily follow that the complement fixation reaction with the cancer antigen will prove to be of clinical value but certainly further study is suggested.

### CONCLUSIONS

By fractionating according to Lehmann-Facins the alcoholic extract of cancer tissue, positive complement fixation reactions were obtained in 70 per cent of 51 cases of carcinoma examined. Of 28 carcinoma serums tested with the ethylene chloride antigen, 100 per cent were positive. With the cholesterolized phosphatid fraction of the alcoholic extract all but 2 of 107 four-plus syphilitic serums reacted positively. With the ethylene chloride fraction, out of 27 four-plus syphilitic serums tested, 11 were negative. Out of 92 normal serums one gave positive reactions with the phosphatid fraction while out of 11 such serums 4 were positive with the ethylene chloride fraction.

The antigen concerned in fixation with cancer serums is not destroyed by heating to 125° C. but is destroyed by certain alkaline reagents. The cancer antigen apparently is different from the syphilitic antigen, and it appears probable that these two antigens may be separated.

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### THE SERUM CALCIUM IN ARTHRITIS\*

EDWARD F. HARTUNG, M.D., AND CARL H. GREENE, M.D., NEW YORK, N. Y.

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WHILE it is generally accepted at the present time that arthritis is a constitutional disease, it is equally true that the most striking clinical manifestations are those in the bones and joints. The ordinary clinical classification into atrophic and hypertrophic forms bears witness to the variety of the osseous changes. In the rheumatoid or atrophic form of arthritis there is a generalized decalcification and rarefaction of the bones involved. This is most marked at the epiphysis but usually involves the cortex of the shaft to a lesser degree. In osteoarthritis or hypertrophic arthritis the process of decalcification is not so marked, but there is a simultaneous deposition of new bone which produces calcified excrescences at the chondro-osseous junctions and eburnation at the denuded areas of epiphysis. It is claimed that the fundamental and initial lesion in osteoarthritis is one of erosion of the articular cartilage and that the osseous changes are secondary and perhaps compensatory in character. There is no question, however, that the metabolism of the bone is altered in both forms of arthritis.

The study of changes in the metabolism of bone is difficult. The mineral balance of the body is not easy to determine, and there have been only a few cases of arthritis in which the calcium balance has been determined satisfactorily. The results in these latter have been, for the most part, inconclusive. This perhaps is to be expected, for arthritis is a chronic disease and a disturbance in the calcium metabolism, too small to be demonstrated conclusively in a balance experiment of a few days' duration, would produce cumulative effects of a marked and extensive character in the course of months or years.

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\*From the Department of Medicine, New York Post-Graduate Medical School and Hospital.  
Received for publication, October 11, 1934.

Further attempts have been made to demonstrate changes in calcium metabolism in arthritis by a study of the calcium content of the blood serum. Previously reported data on this subject have been contradictory and inconclusive. Weil and Guillaumin,<sup>1</sup> Watchorn,<sup>2</sup> Horowitz,<sup>3</sup> and Mark<sup>4</sup> all report the serum calcium to be increased in arthritis and especially in arthritis deformans. Ssamarin,<sup>5</sup> Copp,<sup>6</sup> Funsten,<sup>7</sup> and Morelle<sup>8</sup> report that the serum calcium is normal in many cases, but that it may be elevated in a proportion of the cases especially in osteoarthritis. This proportion varies from an occasional case according to Morelle who not only reported a series of twenty cases but collected many others from the literature, to two-thirds of the cases reported by Ssamarin and Copp. Other investigations as Cajori and Crouter,<sup>9</sup> Jung and Hakki,<sup>10</sup> Bastos and Mazo,<sup>11</sup> Gold,<sup>12</sup> Naehlas,<sup>13</sup> Bauer,<sup>14</sup> or Battistini and Robeechi<sup>15</sup> report that the serum calcium is normal in the various types of arthritis. The determination of the behavior of serum calcium in arthritis is important, for the rôle of the parathyroid glands in regulating calcium metabolism has been fully established and an elevation of the serum calcium is one of the diagnostic points in the syndrome of hyperparathyroidism produced by tumors of the parathyroid glands. Even in the absence of parathyroid tumor various surgeons as Oppel<sup>16</sup> and Ballin<sup>17</sup> have argued that the finding of an elevated serum calcium in a case of arthritis is evidence of hyperactivity of the parathyroid glands and have surgically removed one or more of the latter. This contention has been a source of a great deal of controversy. That there is any relationship between overactivity of the parathyroid glands and arthritis has been denied even more emphatically than it has been affirmed. It should not be affirmed, or parathyroidectomy undertaken, on the basis of a single determination of the serum calcium.

Our own experience has convinced us that the determination of the serum calcium is liable to technical error. If the technique is carefully standardized and rigidly carried out extremely uniform results are obtainable. Unless this is done, however, very different results may be obtained, in different laboratories or even by two technicians in the same laboratory, without obvious analytical error in either case. Because of this factor the demonstration of an elevation of the serum calcium in any disease is much more significant if compared with a series of control determinations made in the same laboratory. Furthermore, as emphasized by Bauer the serum calcium is continuously elevated in cases of parathyroid tumor. A single elevated determination in a series done on one patient, is more likely to be due to technical error than to the transitory parathyroid hyperactivity as reported by Funsten.<sup>7</sup>

Many of the investigators as Cajori and Crouter, Naehlas, and Bauer, who have reported normal values for the serum calcium in arthritis have not reported their data in detail or else the number of cases studied was too small to make the data authoritative. We have, therefore, attempted to answer these questions by a study of the calcium content of the serum in a sufficiently large group of cases of arthritis to permit of a statistical analysis of the results.

## MATERIAL AND METHODS

The subjects for this study were patients in the Arthritis Clinic of the New York Post-Graduate Hospital. A series of fifty cases of rheumatoid arthritis and a like number of cases of osteoarthritis were taken in sequence. Blood specimens were obtained by venipuncture two or three hours after breakfast. The serum calcium was determined by the Clark-Collip Modification of the Kramer-Tisdall Method. The cases therefore all came from one clinic and the determinations were done in one laboratory.

## CONTROLS

The usually accepted normal range of variation for the serum is between 9.0 and 11.0 mg. per 100 c.c.<sup>18, 19</sup> Various authors have reported small groups but the largest control group is that of 852 cases reported by Greene and Boothby<sup>20</sup> from the Mayo Clinic. In the present series we have studied a group of fifty hospital cases in which there was no obvious metabolic or other disturbance which might affect calcium metabolism and a second group of 128 ambulatory cases referred from the private practice of various staff members. These cases were not especially selected and so may be expected to show a greater degree of variability than our first group. The formulas used in the statistical analyses of our results are to be found in the standard textbooks on statistics.<sup>21, 22</sup>

## RESULTS

The serum calcium of fifty cases of rheumatoid arthritis is given in Table I. The mean and standard deviation of this group was  $10.218 \pm 0.699$  mg. per 100

TABLE I  
THE SERUM CALCIUM IN RHEUMATOID ARTHRITIS

CASE	AGE	SEX	SERUM CALCIUM MG. PER 100 C.C.	CASE	AGE	SEX	SERUM CALCIUM MG. PER 100 C.C.
1	27	F	10.2	26	30	F	9.4
2	48	F	10.2	27	34	F	9.6
3	55	F	9.4	28	24	F	9.2
4	51	F	10.1	29	21	F	10.2
5	36	F	9.4	30	25	F	9.8
6	54	F	11.0	31	49	F	9.5
7	44	F	9.8	32	26	F	11.4
8	25	F	10.5	33	28	F	12.1
9	54	F	10.6	34	28	F	10.4
10	32	F	9.8	35	50	F	10.9
11	31	M	10.4	36	61	F	11.1
12	31	F	10.6	37	37	M	12.2
13	48	F	9.6	38	52	F	10.2
14	10	F	9.6	39	53	F	10.4
15	50	F	10.0	40	36	M	10.7
16	58	F	9.6	41	27	F	10.7
17	33	F	9.4	42	68	M	9.4
18	34	M	11.9	43	61	F	10.2
19	64	F	10.8	44	46	M	10.4
20	58	F	10.6	45	36	F	10.2
21	51	F	10.5	46	47	F	9.4
22	28	F	10.7	47	50	F	10.1
23	27	F	10.0	48	51	F	10.2
24	40	M	9.2	49	21	F	9.6
25	41	F	9.3	50	55	M	10.4

c.e. The average age of this group of patients was forty-one years, and 84 per cent were women.

The serum calcium of fifty cases of osteoarthritis is given in Table II. The mean and standard deviation of this group was  $9.986 \pm 0.616$  mg. per 100 c.e. This group of patients was older, for the average age was fifty-two years. Eighty per cent were women.

TABLE II  
THE SERUM CALCIUM IN OSTEOARTHRITIS

CASE	AGE	SEX	SERUM CALCIUM MG. PER 100 C.C.	CASE	AGE	SEX	SERUM CALCIUM MG. PER 100 C.C.
1	40	F	10.0	26	68	F	9.5
2	52	M	10.6	27	52	F	10.6
3	41	F	9.5	28	49	M	9.6
4	58	F	9.3	29	48	F	11.3
5	52	F	9.5	30	50	M	10.6
6	27	M	9.8	31	61	F	9.9
7	53	M	10.8	32	63	M	11.4
8	64	F	9.6	33	62	F	9.9
9	56	F	10.9	34	35	F	9.6
10	62	F	10.6	35	58	F	10.4
11	31	M	9.6	36	52	F	9.8
12	57	F	9.8	37	58	M	11.2
13	48	F	9.2	38	57	F	9.2
14	50	F	9.8	39	63	F	9.8
15	55	F	10.9	40	48	F	9.7
16	46	F	10.8	41	45	F	9.6
17	46	F	9.5	42	49	F	9.7
18	60	F	10.2	43	42	F	9.3
19	46	F	9.7	44	74	F	9.8
20	50	M	11.1	45	69	F	9.8
21	53	F	9.5	46	56	F	9.1
22	45	F	9.8	47	30	F	9.7
23	59	F	10.2	48	53	M	9.1
24	53	F	10.1	49	52	F	10.6
25	53	F	9.0	50	52	F	10.3

TABLE III  
SERUM CALCIUM

TYPE OF CASE	NUMBER OF CASES	MG. PER 100 C.C.			
		MEAN	PROBABLE ERROR OF MEAN	STANDARD DEVIATION	PROBABLE ERROR OF STANDARD DEVIATION
Control					
Greene and Boothby	852	10.241	$\pm 0.015$	0.647	0.010
Control, New York Post- Graduate Hospital					
Hospital patients	50	10.274	$\pm 0.060$	0.622	0.042
Private patients	128	10.231	$\pm 0.046$	0.777	0.033
Rheumatoid arthritis	50	10.218	$\pm 0.067$	0.699	0.048
Osteoarthritis	50	9.986	$\pm 0.059$	0.616	0.042

The statistical analysis of these two groups of patients, together with the control series, is given in Table III. Greene and Boothby<sup>20</sup> found the mean serum calcium of their control group to be  $10.241 \pm 0.647$  mg. The figures for

the control group from this laboratory give almost identical values, namely,  $10.274 \pm 0.622$  mg. per 100 c.c. Using these figures it may be said that approximately 68 per cent of control cases have a serum calcium between 9.6 and 10.9 mg. and 95 per cent between 9.0 and 11.5 mg. per 100 c.c.

#### DISCUSSION

The changes in the serum calcium in arthritis are not marked, for of the whole group of 100 cases studied, the values for the serum calcium were outside of this normal range of 9.0 to 11.5 mg. per 100 c.c. in only three cases. There was no evidence that hyperactivity of the parathyroid glands was a factor in the production of arthritis. The mean serum calcium of the cases of rheumatoid arthritis was  $10.218 \pm 0.699$  mg. This value is practically the same as that found in the control cases.

In osteoarthritis the mean was  $9.986 \pm 0.616$  mg. This value is significantly lower than that found in the control group, the difference between the means being 3.4 times the probable error of the difference. We may, therefore, say with assurance that while individual cases of osteoarthritis show a serum calcium within the normal range, the trend is downward and that in this series of cases the mean value was significantly lowered. The difference between the serum calcium level in cases of rheumatoid arthritis and that in osteoarthritis is further evidence that these two conditions represent different clinical entities.

Our present knowledge of the etiologic factors in osteoarthritis does not afford an explanation for the tendency toward a lowered serum calcium in this condition. The decrease may be due to a fundamental change in the calcium metabolism either as a result of the local lesions or as part of a general constitutional reaction. Before changes in the serum calcium can be considered the specific result of the disease process, however, the influence of various associated factors which may have an effect on calcium metabolism must be excluded.

Age is such a factor, for patients with osteoarthritis usually belong to a somewhat older age group than do those with rheumatoid arthritis. Greisheimer, Johnson and Ryan<sup>23</sup> reported that in adults the serum calcium decreased slightly with age. Also a considerable proportion of older individuals show a decrease in the acidity of the gastric juice. This hypochlorhydria or achlorhydria may reduce the absorption of calcium from the intestine either as a result of changes in the reaction of the intestinal contents or in the permeability of the intestinal wall. Many patients with arthritis are anemic. There is a tendency for the serum calcium to be reduced in anemia either as a result of the anemia or because of a concomitant reduction in the serum proteins.

These and probably numerous other factors may be of importance in producing the slight reduction in the serum calcium in osteoarthritis. At the present time no single factor can be accepted as responsible for the changes noted and much further information is needed before the relative importance of each can be determined.

#### SUMMARY

The values for the mean and the standard deviation of the serum calcium in a group of 50 cases of rheumatoid arthritis were  $10.218 \pm 0.699$  mg. per 100 c.c. These values were essentially the same as those found in control cases.

The values for the mean and the standard deviation of the serum calcium in a group of 50 cases of osteoarthritis were  $9.986 \pm 0.616$  mg. per 100 c.c. The changes in the serum calcium in any individual are perhaps without direct clinical significance, but the trend in the group represents a statistically significant reduction in the mean calcium level. The reason for this change is not obvious. Its presence in osteoarthritis and absence in rheumatoid arthritis are further evidences that these two conditions represent separate clinical entities.

This study of the serum calcium furnishes no evidence that hyperactivity of the parathyroid glands is a factor in the production of arthritis.

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# OCCLUSION OF THE PORTAL VEIN\*

## AN EXPERIMENTAL STUDY WITH ITS CLINICAL APPLICATION

FREDERICK FITZHERBERT BOYCE, B.S., M.D., RALPH LAMPERT, B.S., M.D., AND  
ELIZABETH M. MCFETRIDGE, M.A., NEW ORLEANS, LA.

OUR interest in experimental occlusion of the portal vein arose accidentally, in the course of a study of so-called "liver deaths," as one phase of which we endeavored to produce necrosis of the liver by various forms of interference with its blood supply. In this endeavor we failed, chiefly because all the animals in which complete occlusion was done in a single stage died within a few hours, before such changes could possibly ensue. Our interest in the subject was increased by the appearance, during these experiments, of Elman and Cole's paper on the cause of death in portal occlusion, and we determined to undertake a similar investigation, the results of which, together with their apparent clinical application, we are reporting herewith.

### HISTORICAL DATA

Experimental ligation of the portal vein was first attempted in 1856 and the clinical implications of such a procedure have been recognized for many years, but so little has been written on the subject that it is almost necessary to begin any discussion of it with a brief review of the literature. Much of the historical data which follow are taken from the excellent survey by Neuhof which appeared in 1913.

Oré, in 1856, first demonstrated that ligation of the portal vein in rabbits results in prompt death, his experiments being corroborated on dogs by Claude Bernard in 1858 and by Schiff in 1863. Bernard attempted no explanation of the fatality until 1877, when he advanced the idea that death was caused by exsanguination, which in turn was due to a stasis of blood in the gastrointestinal tract. He apparently overlooked the experimental proof adduced by Tappenheimer five years before, which we ourselves have just confirmed, that more blood can be withdrawn from a dog by venesection without fatal issue than is accumulated in the gastrointestinal tract after portal ligation. Schiff's explanation that death is due to suppression of liver function is no longer tenable, since it has been repeatedly proved that the existence of an Eek fistula is not incompatible with life.

In 1875 Solowieff proved experimentally that animals can survive the ligation singly, at intervals of five to six days, of the superior mesenteric, the gastrophatic and the portal veins, and Neuhof, in 1913, confirmed his findings, al-

\*From the Department of Surgery of the Louisiana State University, School of Medicine.  
Received for publication, October 30, 1934.



though Ito and Omi did not in the single experiment which they reported in 1901. Kusnetzow's demonstration in 1900 that ligation of the portal vein below the entrance of the gastrohepatic vein is fatal, whereas occlusion above this point is not fatal, was not confirmed by any of the last-mentioned workers. Neuhoef, however, proved that gradually induced occlusion of the portal vein is not incompatible with life, and pointed out that under such circumstances there are no changes of consequence in the liver because of the development of a collateral circulation in the gastrohepatic omentum.

The extensive experiments in portal occlusion reported from various Russian clinics are not entirely in accord with the results of the experiments just outlined. Roger, for instance, was able to ligate the portal vein for three hours in a dog and half an hour in a rabbit without serious damage, and Duchinowa, in a large number of cases, found it possible to compress the portal vein for thirty-five minutes with no apparent ill effect. Moreover, the blood studies reported by Tschernikoff and his coworkers were done in many cases on animals subjected to repeated compression and decompression of the portal vein.

Elman and Cole, writing in 1934, conclude that in portal occlusion death can be explained entirely on the basis of a loss of blood from the systemic into the portal area of such magnitude that the blood pressure is reduced to a point incompatible with life and is maintained at this critical level until death occurs. They point out, in support of this hypothesis:

1. That the behavior and appearance of animals subjected to portal occlusion corresponds in all respects with the behavior and appearance of animals who die after blood loss due to frank hemorrhage.

2. That the use of transfusion after portal ligation elevates the blood pressure temporarily and postpones death, even though it cannot avert it.

3. That the same results are achieved by ligation of the aorta above the celiac axis, which prevents blood from entering the splanchnic area.

4. That the engorged intestinal tract cannot aid in supplying loss of fluid, as it can in shock due to other causes.

To consider the question in its clinical aspects, Gintrac, in 1857, collected six cases of pathologic portal occlusion, and a considerable number of such cases have since been reported. The number, furthermore, is undoubtedly greater than is apparent from the literature, partly because, as always happens, many cases are not reported, partly because others are included in papers dealing with other subjects. We recently found, for instance, in a publication by C. H. Peck on pain in the upper abdomen and chest, the report of a case of complete portal stenosis for which operation had been undertaken on a mistaken diagnosis of perforated duodenal ulcer, the patient being alive and well eight years later.

Ransahoff, in 1908, noted that even temporary constriction of the portal vein during operations on the gall ducts causes a sharp and prompt fall in blood pressure, and concluded that if the pressure were continued, the phenomena attending portal ligation in animals would undoubtedly be reproduced. Villard reports the loss of a patient because of temporary pressure on the portal vein with a tampon during an operation for biliary disease. On the other hand

Krymholz reports a personal case in which he compressed the portal vein for eight minutes to check hemorrhage in an incised wound of the liver, and Colp reports that in the cases in which he attempted portal ligation as a deliberate therapeutic procedure for pyelophlebitis of appendiceal origin, tentative compression of the vein, with subsequent occlusion, had no immediate untoward results, although all the cases were eventually fatal. The clinical and experimental observations are at variance in many respects, but it is safe to state categorically that all the clinical evidence goes to prove that gradual occlusion of the portal vein as the result of some pathologic process is not the same as abruptly produced experimental occlusion.

#### EXPERIMENTAL DATA

All our experiments were performed under the same controlled conditions. Dogs were selected for use only after the competency of their renal function had been established, and they were prepared for sixty hours; they were given only milk during the first twenty-four hours and only water during the second twenty-four hours, and they were deprived of all food and fluids during the final twelve hours. They were weighed immediately before operation, and they were anesthetized either with intravenous veterinary nembutal (kindly supplied to us by the Abbott Laboratories) or a light ether narcosis.

*Series I.*—A. Rapid evisceration was done through a midline incision, and the eviscerated mass, which consisted of the entire gastrointestinal tract from the cardiac end of the stomach to the anus, together with the pancreas and the spleen, was ligated, to guard against loss of blood and intestinal contents. This mass was then weighed, to determine its relation to the body weight of the animal. The average in seven dogs was 6.87 per cent.

B. Through a midline incision an aluminum band was placed on the portal vein just below its bifurcation. Seven dogs were used. Profound shock was promptly evident in them all, and death followed in from thirty-three to one hundred thirty-four minutes, the average duration of life being eighty-seven minutes. Immediately after death evisceration was done, by the method just described, and the eviscerated mass was weighed and its weight checked against the body weight. The proportion ranged from 7.98 per cent to 10.9 per cent, the average being 9.92 per cent, in contrast to the average normal proportion of 6.87 per cent. The loss of blood into the intestinal tract, therefore, varied from 1.11 per cent to 4.03 per cent of the body weight, with an average of 3.05 per cent in the seven dogs.

In all cases there was an insignificant rise in the leucocyte count after operation, but no other changes in the total and differential blood count or in the blood chemistry. The clotting time showed a decrease of from ten to thirty-five seconds within the first half hour after operation, a finding which we shall discuss in more detail later.

C. In one dog portal occlusion was done by the method described, following which 685 c.c. of glucose solution were given intravenously. This dog lived for three hours and ten minutes and was the only animal subjected to portal occlusion which was able to stand up after operation or which showed any recovery from

his shock. Such a measure, like the transfusions reported by Elman and Cole, can evidently postpone death temporarily if it is promptly invoked. At autopsy this dog had 250 c.c. of fluid in the peritoneal cavity, a finding which was noted in no other animal in the series.

D. A control series of dogs were bled from 4 to 5 per cent of their body weight, an average of 4.56 per cent. In some instances the venesection was done in one stage, in others it was spaced, to correspond with the average duration of life after portal occlusion. In no case was marked shock evident, and in no case did death follow the procedure. Another dog, however, which was bled 5.8 per cent of his body weight at one time, exhibited profound shock and died within ninety minutes.

*Series II.*—A. Two dogs subjected to occlusion of more than two-thirds of the portal vein in one stage died within three and a half hours and eighteen hours, respectively.

B. One dog was subjected to occlusion of the right portal vein, followed within a week by occlusion of the left portal vein. A second dog was subjected to occlusion of the left portal vein, followed within ten days by occlusion of the right portal vein. Both dogs died within two hours of the second operation.

C. In one dog half of the portal vein was occluded, then three-quarters, and then the entire vein, at intervals of five to seven days. In the second dog occlusion of two-thirds of the vein was done at the first operation and the occlusion was completed a week later. In neither animal was there any evidence of shock and both were to all appearances perfectly normal when they were sacrificed.

Five weeks after the last operation the abdomen was opened and the portal vein was injected below the point of obstruction, in one case with lead chromate, in the other with a solution of carmine treated with ammonia in gelatin. In both animals an abundant collateral circulation could be traced through three distinct venous channels:

1. Gastric to esophageal.
2. Duodenal and colic to left renal.
3. Inferior mesenteric to hemorrhoidal to hypogastric.

*Series III.*—Simultaneous ligation of the portal vein and of the gastric, splenic, and inferior and superior mesenteric arteries, with the idea of preventing part of the blood from entering the portal system, was followed by death within ninety minutes. Elman and Cole, in a similar experiment, were able to keep their animals alive more than six hours by the use of transfusion, and noted that less blood was required to achieve this result than when arterial ligation was not done.

From these three groups of experiments we have drawn the following conclusions:

1. Occlusion of the portal vein in a single stage is invariably fatal, as is successive occlusion of the portal branches or the occlusion of more than two-thirds of the vein at any one time.

2. Stage occlusion, as Neuhof originally proved, is not incompatible with life because this method permits the development of an abundant collateral circulation which takes over the task of the portal circulation.

3. The explanation advanced by Elman and Cole for the cause of death in portal occlusion, that the loss of blood into the gastrointestinal tract produces a fall in the blood pressure to a point incompatible with life, is not acceptable. That the blood loss plays an important part in the fatal issue cannot be denied, but that it is the only cause of death, or even the principal cause, we do not believe, for we have proved, as Tappenheimer proved in 1872, that in order to cause death, the animals must be bled by venesection far beyond the blood loss into the intestinal tract after portal occlusion. Furthermore, we do not believe that the relatively abrupt fall in blood pressure after portal occlusion is likely to be caused by the relatively slow loss of blood into the intestinal tract. Finally, an additional argument against the theory is the fact that the greatest volume of blood which reaches the right heart by way of the inferior vena cava does not come from the liver.

4. Our own theory is that death after portal obstruction is to be explained in two ways, the first explanation having to do with the initial fall in the blood pressure, the second with its maintenance at a level incompatible with life. The initial fall, in our opinion, is brought about by the same neurogenic factor, with the same resulting inhibition, as is present in primary shock, plus the abrupt shunting of a large amount of blood from the general circulation, which implies, in turn, a corresponding decrease in the volume of the circulating blood. The maintenance of the blood pressure at the critical level is also due to two things. In the first place, there is a continued gradual withdrawal of more and more blood from the general circulation into an area from which it cannot escape, with a corresponding gradual decrease in the volume of the circulating blood; in the second place, there is a stagnation in the intestinal tract of the blood thus diverted, which has the same effect as the loss of the same amount of blood would have in any other hemorrhagic condition. The neurogenic factor, it seems to us, is amply established by the experiments of Thole, who demonstrated that when the vagus nerve is sectioned before portal occlusion is done, the drop in the blood pressure is more gradual and the duration of life is longer, though death is just as inevitable. The decerebration experiments of various other Russian investigators are confusing rather than helpful, it seems to us, because the procedure of decerebration in itself introduces factors which hopelessly cloud the issue.

5. The decrease in the clotting time of the blood which we observed in our experiments is entirely in keeping with the changes reported by Russian investigators following portal ligation. The decrease is more notable terminally than initially, but we did not carry our studies beyond the first half hour after operation, because their work is so complete. In all the cases which they report the decrease was very marked; in one case reported by Tschernikoff and his co-workers, for instance, the clotting time fell from three minutes ten seconds before operation to fifteen seconds within eighty minutes after operation. There was al-

ways a prompt return to normal figures when only temporary ligation was done, but the longer the compression had endured the slower was the return to normal.

Why this decrease should occur is not clear. The original observation was made accidentally, when it was noted that after portal occlusion the cannulas in use promptly became choked with clotted blood. Liver changes do not seem responsible, for death occurs too promptly after operation for the liver substance to be seriously affected. The theory that the spleen delivers into the circulation certain products which tend to lower coagulability does not hold, for in some experiments the splenic circulation was tied off before portal occlusion was done. Detailed morphologic and chemical blood studies revealed no significant blood changes. Brandes and Simonds' explanation of a similar decrease in the clotting time after mechanical constriction of the hepatic vein, that the heparin in the blood is used up too rapidly, cannot be accepted here, for the source of the supply of heparin is not altered by portal occlusion. The speculative explanation advanced by Tschernikoff and his coworkers, that some obscure functional disturbance of the intestine is responsible, is at least reasonable, for the effect of the products of intestinal metabolism under such circumstances is undoubtedly very important. Their results are as yet inconclusive, and we propose to continue our own investigations along the line they have suggested and to study the effect upon the blood of lymph obtained from the gastrointestinal tract by way of the thoracic duct.

#### CLINICAL CONSIDERATIONS

The idea of ligation of the affected vein for pyemia of various origins is no new thing. John Hunter did it successfully on the saphenous vein in 1784, and in the following century many similar attempts were reported, though all of them did not end so well. General interest in the operation, however, was not aroused until Zaufel, in 1894, suggested its application to the internal jugular vein in pyemia of otitic origin and Viereck, seven years later, reported 108 such cases with recovery in 89.

The operation was next applied to puerperal pyemia. In 1894 and in 1898 Sippel and Freund had proposed treating this disease by excision of the affected pelvic veins, in addition to hysterectomy, but the mortality of the latter procedure was in itself so appalling that their suggestion won little attention and less approval. In 1902, however, Trendelenburg and Bumm published their results with simple ligation of the pelvic veins in puerperal pyemia, and Miller's comprehensive study in 1917 established beyond doubt the wisdom of this procedure in the occasional properly selected case. The latter's collected statistics show a gross mortality of 51.6 per cent, and a corrected mortality, for properly chosen cases, of 33.9 per cent, and there can be no question that such a reduction in the mortality of a disease which, untreated, exhibits a death rate of 60 to 70 per cent, warrants earnest consideration of the method which has brought it about.

The theory of appendiceal and appendicitis:

of a similar procedure in pyelophlebitis of appendicitis. Zaufel was the first to point out. That appendicitis in this condition is not nearly

so important a consideration as the fact that pylephlebitis is a complication of acute appendicitis in a definite, although small, percentage of all cases, especially in old people, and the fact that it is variously estimated to be responsible for from 5 to 10 per cent of all fatal cases. Moreover, while recoveries after it are recorded, although many of the reported cases leave one with the impression that the cure occurred when the existence of the complication was never proved, the mortality is still enormous. It was 59 per cent in the 53 cases collected by Eliason, who comments, with reason, upon the wide discrepancy between the 20 recoveries in the 23 cases collected by Schlesinger (which are included in his 53 cases) and the 7 recoveries in the 30 cases which he himself collected. It seems scarcely necessary to point out that the latter figures are far more in keeping with the experience of most surgeons than are the former.

In a disease of such gravity heroic measures are undoubtedly justified, but one must be certain, before adopting them, that they are not too heroic. In the first place, the operation is frequently done too late even when it is done immediately, for thrombophlebitis may be coincident with the clinical onset of the disease, as in the case mentioned by Keyes, in which death occurred from this complication four days after the removal of an acutely inflamed but nonsuppurative appendix. In the second place, although the fundamental pathologic process is the same and the fundamental principle of treatment is the same, there is an even wider difference between ligation of the portal vein and ligation of the pelvic veins than between ligation of the pelvic veins and ligation of the internal jugular vein. Venous ligation in an area drained by one vein and a similar procedure in an area drained by many veins are by no means the same thing. In the third place, as we have already pointed out, there is a wide difference between gradual occlusion of the portal vein by a slow pathologic process, and its abrupt mechanical occlusion by a surgical procedure. Finally, and most important, all the experimental evidence and most of the clinical evidence forces one to the conclusion that portal occlusion is either entirely incompatible with life or introduces so serious a degree of risk that the remedy threatens to be even worse than the disease for which it is invoked.

But the remedy has been tried. Beer, following Neuhof's suggestion, undertook deliberate ligation of the portal vein in pylephlebitis of appendiceal origin, and as the first step attempted to anastomose the spermatic vein with a branch of the inferior mesenteric vein, with the idea of increasing the collateral circulation. When the attempt failed he resorted to omentopexy, which was included in Neuhof's original suggestion, and three days later performed cholecystectomy and portal ligation. The patient's jaundice promptly decreased, but he died within forty-eight hours.

In 1926 Colp reported four fatal cases of ligation of the portal vein, one of which can be promptly discarded, since, because of the distortion caused by adhesions, the superior mesenteric vein was ligated in the mistaken belief that the ligature was being placed on the portal vein.

Of the other three patients, one was moribund at operation and died within three hours, the second lived two days, and the third, in whom occlusion was done in two stages, died six days after the second operation. Autopsy in two

ways a prompt return to normal figures when only temporary ligation was done, but the longer the compression had endured the slower was the return to normal.

Why this decrease should occur is not clear. The original observation was made accidentally, when it was noted that after portal occlusion the cannulas in use promptly became choked with clotted blood. Liver changes do not seem responsible, for death occurs too promptly after operation for the liver substance to be seriously affected. The theory that the spleen delivers into the circulation certain products which tend to lower coagulability does not hold, for in some experiments the splenic circulation was tied off before portal occlusion was done. Detailed morphologic and chemical blood studies revealed no significant blood changes. Brandes and Simonds' explanation of a similar decrease in the clotting time after mechanical constriction of the hepatic vein, that the heparin in the blood is used up too rapidly, cannot be accepted here, for the source of the supply of heparin is not altered by portal occlusion. The speculative explanation advanced by Tschernikoff and his coworkers, that some obscure functional disturbance of the intestine is responsible, is at least reasonable, for the effect of the products of intestinal metabolism under such circumstances is undoubtedly very important. Their results are as yet inconclusive, and we propose to continue our own investigations along the line they have suggested and to study the effect upon the blood of lymph obtained from the gastrointestinal tract by way of the thoracic duct.

#### CLINICAL CONSIDERATIONS

The idea of ligation of the affected vein for pyemia of various origins is no new thing. John Hunter did it successfully on the saphenous vein in 1784, and in the following century many similar attempts were reported, though all of them did not end so well. General interest in the operation, however, was not aroused until Zaufel, in 1894, suggested its application to the internal jugular vein in pyemia of otitic origin and Viereck, seven years later, reported 108 such cases with recovery in 89.

The operation was next applied to puerperal pyemia. In 1894 and in 1898 Sippel and Freund had proposed treating this disease by excision of the affected pelvic veins, in addition to hysterectomy, but the mortality of the latter procedure was in itself so appalling that their suggestion won little attention and less approval. In 1902, however, Trendelenburg and Bumm published their results with simple ligation of the pelvic veins in puerperal pyemia, and Miller's comprehensive study in 1917 established beyond doubt the wisdom of this procedure in the occasional properly selected case. The latter's collected statistics show a gross mortality of 51.6 per cent, and a corrected mortality, for properly chosen cases, of 33.9 per cent, and there can be no question that such a reduction in the mortality of a disease which, untreated, exhibits a death rate of 60 to 70 per cent, warrants earnest consideration of the method which has brought it about.

The theoretic possibility of a similar procedure in pyelphlebitis of appendiceal origin is obvious, as Nienhof was the first to point out. That appendicitis is the most frequent antecedent disease in this condition is not nearly

4. The theory is advanced that death in portal occlusion is due:

a. To an abrupt and immediate fall in blood pressure, brought about by a neurogenic factor with resulting reflex inhibition, as in primary shock, plus the abrupt diversion of a large amount of blood from the circulation, with a resulting decrease in blood volume.

b. To the maintenance of the blood pressure at this critical level, as the result of a continued gradual withdrawal of blood from the circulation, with a continued gradual decrease in blood volume as the diverted blood is collected in an area from which it cannot escape, plus the stagnation of this blood in the gastrointestinal tract, which has all the effect of primary hemorrhage.

5. Occlusion of the portal vein for pylephlebitis of appendiceal origin is discussed in its clinical aspects, and it is concluded that the operation, although theoretically sound and technically possible, actually has a very limited field and always is attended with very grave risks.

NOTE.—This work was done with the cooperation and approval of Dr. Urban Maes, Director of the Department of Surgery of the Louisiana State University, School of Medicine. Our thanks are due to Dr. D. D. Baker, of the Department of Anatomy, who demonstrated the collateral circulation for us, and to Dr. W. B. Wright, of the Interns Staff of Charity Hospital, who assisted us in many of the experiments.

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cases showed hepatic insufficiency, which the author, probably correctly, attributes to the suppurative process rather than to the operative procedure. In both cases an adequate collateral circulation was present, which means, Colp points out, either that a beginning thrombophlebitis of the portal vein opens up collateral veins, or that the hepatopetal veins in some individuals are competent to take over the circulation immediately when sudden occlusion occurs.

It should be mentioned here that detailed studies of the collateral circulation were made by Charpy in 1898 and by Pick in 1909, the original studies having been made by Frerichs in 1861. Pick reports a particularly striking case in which the portal vein had been occluded for years by a cavernoma of the vessel wall, but in which the portal blood was carried to the liver by veins in the gastrohepatic omentum. The existence of this set of veins was pointed out originally by Charpy, who coined for them the term hepatopetal.

Colp's and Beer's cases, unsuccessful though they were, at least proved that in the human being death does not follow portal occlusion as promptly as it does in the experimental animal, and the same conclusion can be drawn from the cases of ligation of the portal vein for injuries of the liver which were reported by Brewer in 1908 and by Hallopeau in 1910. Furthermore, in pylephlebitis of appendiceal origin conditions are more favorable for portal occlusion than they are in the experimental animal, since there is always present before the occlusion some degree of venous obstruction. It would seem, therefore, on the basis of both clinical and experimental evidence, that if the occlusion has involved a third or more of the lumen of the vein, it might be justifiable to risk the surgical completion of the occlusion. If the occlusion has not progressed to this point, however, or if one branch of the vein is already occluded, the procedure introduces an element of risk even greater than the risk inherent in the untreated disease. In other words, the operation, however sound may be the theory upon which it is based, has necessarily a very limited field, and it would be well for the surgeon who proposes to undertake it to remember that Gerster's dictum, uttered in 1903, still cannot be gainsaid, that while exposure and evacuation of the thrombosed veins is always highly desirable, the procedure is always difficult and is usually impossible.

#### SUMMARY AND CONCLUSIONS

1. A brief résumé is given of previous experimental and clinical work on portal occlusion.
2. A series of experiments is reported which prove:
  - a. That complete occlusion of the portal vein in one stage, as well as successive occlusion of both branches, is incompatible with life.
  - b. That occlusion of the main portal trunk in several stages is a feasible procedure if not more than two-thirds of the vein is occluded at any one time.
3. Experimental proof is advanced to disprove the theory that the loss of blood into the gastrointestinal tract is the only cause or the chief cause of death in portal occlusion.

## CONCLUSIONS

1. Animals on an oat diet are definitely more sensitive to the ethylhydrocupreine than the animals on a mixed diet.
2. Animals on carrot diet reacted less severely than the animals on mixed diet.
3. It is quite probable that the differences in reaction of the animals to this alkaloid are due to difference in the acid-base balance of the system, as evidenced by the difference in the urinary reaction.

It is my pleasant duty to express sincerest gratitude to Dr. Bernard Fantus, for his suggestions and criticisms proved of inestimable value.

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FAT EMBOLISM<sup>\*</sup>

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A CONTROL STUDY OF BLOOD SERUM AND URINE

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FRANK J. JIRKA, M.D., AND CARLO S. SCUDERI, M.D., CHICAGO, ILL.

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THE diagnosis of fat embolism in fractures offers many difficulties from a clinical viewpoint. The symptoms of this condition being more or less irregular and somewhat indefinite, depending purely on the local phenomena produced by the liberated fat droplets, leaves the clinician to prove the diagnosis by one of the laboratory methods. As the quantitative fat determinations of the blood are very complicated, and the variations in the normal so great, this method at the present time is of little value. The presence of fat droplets in the sputum, Warthin's sign, is present in numerous other chest conditions so it cannot be considered reliable. This leaves us with but the examination of the urine and blood serum, in these cases, to substantiate such a diagnosis.

As far as we have been able to determine from the literature the study of the urine and blood serum in normal cases, and fresh fractures, has never been done. For this reason the following work has been carried on to see whether or not free fat is found in normal control cases.

Two hundred miscellaneous hospital cases were used for the urine examination. In this series only ambulatory male patients were used because of the greater ease in collecting the urine specimen. Great care was exercised in this work, to have the patient completely empty the bladder, as the authors have shown in a recent article,<sup>1</sup> that unless all of the urine of the bladder is collected, the floating fat droplets will not be expelled, and hence the urine examination would convey the wrong conclusions.

The urine specimens were collected and placed in a cool place overnight. The next morning the surface of the urine was carefully examined for floating

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<sup>\*</sup>From the Surgical Service of the Cook County Hospital, and the Department of Surgery, University of Illinois.

Received for publication, October 31, 1934.

# INFLUENCE OF DIET UPON THE TOXICITY OF ETHYLHYDROCUPREINE HYDROCHLORIDE\*

## PRELIMINARY REPORT

A. J. NEDZEL, M.D., CHICAGO, ILL.

IN A PREVIOUS study† we found that the animals on carrot or mixed diet reacted to the injection of cocaine more or less similarly; though the animals on the carrot diet reacted more vigorously. The animals on water and oats diet exclusively were definitely more sensitive to cocaine poisoning. In general, in the oat-fed animals, the toxic effects of the cocaine were definitely greater and recovery was considerably delayed. In continuation of this study we have undertaken a new series of experiments, presented here, replacing cocaine with ethylhydrocupreine hydrochloride.

The observations were conducted on 55 rabbits, divided into 3 groups. Each group was kept for two to three weeks on special diets. In the first group the animals (15 rabbits) were fed oats, carrots, cabbage, alfalfa and water (as standard normal diet); the second group (20 animals) was fed water and oats, and the third (20 animals) on carrots exclusively. During the experiment the urine of the animals was tested daily for its reaction (Folin's method). At the end of the first week the urine of the rabbits of the first group was slightly alkaline or acid, although more generally alkaline; the urine of the second group (oat diet) showed distinct and stable acid reaction (up to 90); and that of the third (carrot diet) group was persistently alkaline.

The experiments consisted in injections of ethylhydrocupreine hydrochloride into the marginal ear vein of a rabbit. The injections were performed uniformly, the duration of injection being about ten seconds. The dose of ethylhydrocupreine was 18 mg. in 1 c.c. of water per kilo of animal's weight. After the injections the animals were observed for forty-eight hours, and their death or survival recorded. The deaths in the greater majority of cases occurred in from one to three minutes after the injection of the ethylhydrocupreine.

The results of experiments are presented in Table I.

TABLE I

DIETS	DIED	SURVIVED	TOTAL NO. OF ANIMALS	MORTALITY PER CENT
General diet (first group)	8	7	15	53
Oat diet (second group)	12	8	20	60
Carrot diet (third group)	8	12	20	40

\*From the Department of Bacteriology and Preventive Medicine and the Department of Pharmacology, University of Illinois, College of Medicine.

Received for publication, October 31, 1934.

†Nedzel, A. J.: J. LAB. & CLIN. MED. 19: 875, 1934.

## CONCLUSIONS

1. Control cases in the study of fat embolism revealed no free fat in either the blood serum or urine specimens.
2. Care should be used completely to empty the bladder of suspected cases, otherwise the floating fat will escape expulsion, if present (Jirka and Seuderi).
3. All questionable films and débris should be stained with Sudan III for final classification.
4. We were unable to confirm the findings of Riedel, that fatty casts were frequently found in the urine of fresh fracture cases.
5. Needles kept in oily substances should never be used for blood collection, if the results are to be relied upon.

The fracture cases are from the service of Dr. Wm. R. Cubbins. The authors wish to express their gratitude.

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## LOCAL ADRENALIN EFFECT AFTER SYMPATHECTOMY\*

## I. THE PERIPHERAL VESSELS: A PRELIMINARY REPORT

ARTHUR M. WRIGHT, M.D., JOHN H. MULHOLLAND, M.D., K. LEORA  
McCLOSKEY, M.D., AND FRANK WANG CO'TUI, M.D.,  
NEW YORK, N. Y.

THE increased sensitivity to adrenalin which certain tissues acquire after those tissues have been deprived of their sympathetic nerve supply is known.

The best example is probably that sensitization which occurs in the denervated heart as shown by Elliot in 1905.<sup>1</sup> Auer and Meltzer had, in 1904,<sup>2</sup> remarked the increased sensitiveness of the denervated iris. Anrep<sup>3</sup> demonstrated that denervation causes the blood vessels in the ear of a rabbit to respond to dilutions of adrenalin as high as 1 to 250 million, a phenomenon which may be put to use in the assay of small quantities of adrenalin. Cannon<sup>4</sup> has used an isolated strip of intestinal muscle to assay and detect the presence of adrenalin in the blood. Recently Freeman, Smithwick, and White<sup>5</sup> reported evidence of sensitization of peripheral vessels in human patients who have been sympathectomized for Raynaud's disease. They used intravenously administered adrenalin or endogenous adrenalin secreted in insulin hypoglycemia.

During the course of some experiments in this laboratory on sympathectomized dogs, adrenalin was injected subcutaneously in a sympathectomized area

\*From the Laboratory of Experimental Surgery, New York University and Bellevue Hospital Medical College.

Received for publication, November 2, 1934.

fat droplets. Any questionable droplets were removed, stained with Sudan III and examined microscopically. In this entire series not one case showed any free fat.

The Serology Department of the Cook County Hospital was kind enough to permit us to have the unlimited use of the blood serums that were sent to them for Wassermann tests. Each specimen was labeled, centrifuged and then placed in the refrigerator overnight. The following morning each tube was carefully examined. All questionable droplets or débris were stained and examined microscopically. On five occasions definite fat droplets were found floating on the surface. However, our findings proved only that the needles had been kept in liquid petrolatum in each case, and evidently the boiling did not dislodge the oil from the inside of the needle. When the blood was drawn the oil found its way into the specimen. Eleven of these cases showed a chylous emulsion which rapidly found its way to the surface. This is physiologic as any blood serum drawn within an hour or two after the ingestion of any fatty material presents this phenomenon. In each of the above cases, the above contention was found to be correct.

Now that we had to our own satisfaction been able to determine what non-traumatic cases showed, we studied fifty fresh fracture cases.

Only male patients, suffering from major fractures, were studied. The duration of the fractures was from one to seven days. The urine was collected by having the patient either in the sitting or standing position, and then having him completely evacuate the bladder. Ten cubic centimeters of blood were then drawn from the arm and both specimens were centrifuged and examined. The urine residue was stained with Sudan III, and examined microscopically. Occasionally casts were found, but in not one instance were we able to find fatty casts such as Riedel<sup>2</sup> described as being present in the urine of 42 per cent of fresh fracture cases.

The casts were no more numerous, nor in any way different, than the casts found in any group of urine specimens.

Not one specimen of blood serum or urine showed any evidence of free fat droplets.

For a résumé of these cases refer to Table I.

TABLE I

<i>Type of Fracture:</i>		<i>Number of Hours Since Accident:</i>	
Tibia	20	1- 24 hours	11
Femur	14	24- 48 hours	8
Humerus	7	48- 72 hours	6
Fibula	5	72- 96 hours	9
Patella	1	96-120 hours	6
Forearm both bones	1	120-144 hours	3
Pelvis	1	144-168 hours	7
Ribs	1		—
Total cases	50	Total cases	50
<i>Number of Cases Showing Fat:</i>		<i>Number of Cases Showing Urine Casts:</i>	
Urine	0	9 cases from 1-2 to 1-12	
Blood serum	0	L.P.F. in centrifuged specimens.	

The finding is reported here for two reasons: Sympathectomy has become a more or less popular surgical procedure for a variety of conditions. The danger in injecting adrenalin into the arm of a patient who has had a cervical sympathectomy is a real one.

Gask, in a recent issue of the *British Journal of Surgery*,<sup>6</sup> casts some doubt on the uniform excellence of results heretofore claimed for sympathectomy in Raynaud's disease. In following cases for over a year in which there had been an immediate and dramatic improvement at operation, he noticed a return of symptoms. Inability of the organism to adjust for heat and cold through the vascular system may not be the whole explanation.

#### SUMMARY AND CONCLUSIONS

1. Tissues supplied by nerves from the thoracolumbar division of the autonomic nervous system when deprived of these nerves tend to become hypersensitive to adrenalin. This is true of the iris, the heart, intestinal musculature, and is now shown to be true in peripheral vessels.

2. Peripheral tissues, when the arterial supply is sympathectomized, undergo necrosis on the injection of ordinary doses of moderate dilutions of adrenalin subcutaneously, in the dog.

3. The time of the onset of sensitization and the possible relation to peripheral vascular disease is being studied.

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of two dogs. Dry necrosis occurred at the site of injection in both. One of these dogs died. The occurrence of abscess and gangrene is not an uncommon complication of adrenalin administration intramuscularly or subcutaneously in animals and in man, and is usually attributed to individual idiosyncrasy or infection. However, in the case of these two dogs, the possibility exists that the pathologic changes might have been caused by a sensitivity of so marked a degree that adrenalin in the concentrations given causes such a prolonged and pronounced vasoconstriction as to result in ischemic necrosis. This being the case, the increased sensitivity demonstrated by the above mentioned authors is of pathologic as well as of pharmacologic and physiologic interest. It was decided, therefore, to study this phenomenon under stricter experimental conditions than those which governed in the case of these two dogs.

Four animals were prepared by bilateral lumbar sympathectomy. Thus the arteries of the hind legs were denervated of their autonomic supply.

One cubic centimeter of 1 to 1,000 adrenalin solution was injected into the subcutaneous tissues of the hind legs of two of these dogs, and 0.5 c.c. of 1 to 1,000 adrenalin solution was similarly injected into the hind legs of the other two dogs. Aseptic precautions were taken. As controls, the same quantity of physiologic saline solution was injected into a nearby area within the sympathectomized region of each of the four dogs and the same quantity of adrenalin was injected into the subcutaneous tissues of one of the forelegs, which were of course normally supplied with sympathetic nerve fibers. All the needles and syringes used were sterilized and handled under the same conditions.

In every instance where the adrenalin was injected into the sympathectomized leg, a definite area of necrosis developed within twenty-four hours. At the onset all these areas of necrosis were typical. They were circular, sharply demarcated, and dry. On about the third day separation of the necrosed tissues occurred, and in two animals the necrosis later became extensive. The appearance of this late necrosis suggested that adrenalin had seeped to a more dependent level producing necrosis 3 to 5 inches below the point of injection. In none of the other sites of injections for control, saline or adrenalin, was there any evidence of disturbance at any time after the injection.

All these animals were injected after a period of at least eight days had elapsed following sympathectomy. The time at which this sensitization occurs has not been accurately determined. Freeman, Smithwick, and White state that a latent period of six to eight days must pass before the sensitization is manifested in the peripheral arteries. In the case of the iris and isolated intestinal muscle the sensitization seems to be immediate. In one other dog adrenalin was injected at the time of operation and twenty-four hours later. The dog died in forty-eight hours from pneumonia and at that time there were no evidences of gangrene, showing in this case at least, the sensitization does not occur in the first twenty-four hours. This method of determining the time of sensitization has been abandoned and a different and more delicate type will have to be used. We are also investigating the possible relation between this phenomenon and the changes which occur in certain functional vascular disorders.

was found to be packed into a volume of about 0.05 c.c. The broth was drawn off with a pipette. To one tube was added enough vesicle fluid to make a total volume of 10 c.c., to a second tube was added a similar volume of blood serum, and to a third tube a like amount of distilled water. The tubes were shaken until homogeneous suspensions were obtained.

Tests were made by placing 0.5 c.c. of organism suspension in each of a series of sterile tubes and adding to each, 1 c.c. of the particular drug solution being used. At the end of fifteen-minute intervals transfers were made to broth tubes which were incubated for seventy-two hours before readings were made. Tests were made in the above manner with the organisms suspended in distilled water (control), in blood serum, in vesicle fluid, and also with the original broth suspension.

Hide powder, being a solid, had to be handled in a slightly different manner. Portions of hide powder (0.075 g.) were weighed into each of a series of sterile test tubes. It was found to be very difficult to sterilize the hide powder and still have a material left which could later be put into a more or less homogeneous suspension. This was finally accomplished by heating the tubes containing the hide powder at 55° C. for three separate periods of two-hours, one and one-half hours and thirty minutes, respectively.

When tests were made 1 c.c. of a distilled water-organism suspension was added to each tube of hide powder. The resulting suspension was stirred with a transfer needle in order to obtain as near a homogeneous suspension as possible. Then 2 c.c. of drug were added and tests made as before.

The amounts of solid material in each test solution were approximately the same. The vesicle fluid was found to contain 0.075 g. of total solids per c.c.; cow blood serum, 0.087 g. per c.c.; and the broth solution 0.065 g. per c.c. The hide dust was weighed out so that each cubic centimeter of test suspension contained approximately 0.075 g.

#### DISCUSSION

The results of the tests are shown in Table I. In it are noted the type of suspension and the highest dilution of the drug effective against a particular suspension. Where growth occurred even in the presence of a saturated solution of a given drug, it is shown by the word "none." The approximate dilution representing saturation of such drugs is given in a footnote at the bottom of the table.

Generally considered the results noted in the table reemphasize the possible discrepancies that may exist between promising laboratory experimentation and successful clinical results. As noted in the previous paper<sup>1</sup> iodine surpasses all other drugs tested when used against water and broth suspensions. Its power to kill, however, is greatly reduced in the presence of proteins. An effectiveness up to 1:75,000 in water suspension and 1:20,000 in broth suspension is successively reduced to 1:5,000, 1:2,000 and 1:1,000 in the presence of hide dust, vesicle fluid, and blood serum, respectively. N-hexyl resorcinol, on the other hand, while less effective on water and broth suspensions is not so drastically affected by the introduction of proteins. Compared to an effective dilution of



# THE FUNGICIDAL POWER OF PHENOL DERIVATIVES\*

## II. STRENGTH IN THE PRESENCE OF PROTEINS

GLENN J. WOODWARD, M.A., PORTLAND, ORE., WITH LYLE B. KINGERY, M.D.  
AND ROGER J. WILLIAMS, PH.D., D.Sc.

IN AN earlier article<sup>1</sup> we reported the results of a series of studies made of the effect on the fungicidal power of certain phenol derivatives of the introduction of various alkyl groups and halogens. It has long been recognized that there is often a wide difference between results obtained in the test tube and successful clinical application. Of the possible factors responsible for this difference two are perhaps preeminent; i.e., first, the physical difficulty of delivering an effective quantity of a given drug to the site of the infection; second, a tendency on the part of the drug applied to combine more freely with the epidermal structures present than with the organisms growing therein. In other words, assuming a certain amount of penetration on the part of the remedy applied, there still remains the requisite of ability to kill or restrain in the presence of tissue proteins completely surrounding the invading organism.

The present work was undertaken in the hope of gaining some information concerning the activity of a group of drugs when used in the presence of certain proteins. For this three of the more active phenols were compared with iodine and four other drugs of common clinical usage. The choice of proteins to be used offered certain difficulties. After considerable preliminary work those chosen consisted of human vesicle fluid, blood serum, and hide dust. The vesicle fluid was obtained from vesicles induced by cantharides plasters, the fluid being withdrawn aseptically and kept in sterile receptacles. Blood was obtained in the usual manner and the supernatant serum decanted. The hide dust was the ordinary commercial product employed by leather manufacturers in the titration of tannic acid solutions. According to information relative to its preparation the keratin and keratohyalin elements present are preserved sufficiently intact to serve the purpose of these estimations. Its use in preference to that of human scales, as in the work of Legge, Bonar and Templeton<sup>2</sup> was prompted by a desire to demonstrate any possible presence or absence of selective action on the part of drugs given the opportunity of reaching both organism and protein in comparable concentration.

### PROCEDURE

Ten cubic centimeter portions of broth suspension of *Monilia tropicalis* which had been growing for seventy-two hours at 37.5° C. were centrifuged for five minutes at 1,000 revolutions per minute. After being centrifuged the yeast

\*From the Department of Chemistry, Oregon State College, Corvallis, and Department of Dermatology, University of Oregon Medical School.  
Received for publication, October 12, 1934.

serum and other natural protein solutions were only partially successful. Better results were had by using commercial peptone mixtures. Further work along this line will be done later.

The results also seem to show that some of the higher phenol derivatives deserve more clinical study in the treatment of fungus diseases than they have had thus far.

#### SUMMARY

1. Three phenol derivatives have been compared with iodine and four other drugs of common clinical usage in the presence of blood serum, vesicle fluid, and hide powder.

2. The fungicidal power of iodine as determined in water and broth suspensions was greatly reduced in suspensions of serum, vesicle fluid, and hide powder; that of *n*-hexyl resoreinol was reduced but the relative diminution was less than that of iodine; saturated solutions of chlorothymol failed to kill in the presence of proteins while thymol was effective only in the presence of hide powder; benzoic acid, salicylic acid and sodium thiosulphate were entirely ineffective in the protein suspensions, but sodium hypochlorite maintained its killing power in high concentrations.

3. Higher phenols have been found worthy of further clinical study in the treatment of fungus diseases.

4. A drug more specific in its action than those now available is needed.

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1:20,000 and 1:15,000 in water and broth suspensions, it retains its effectiveness in dilutions of 1:5,000, 1:2,000, and 1:2,000 in the presence of the three substances used. Not only is its relative diminution less than that of iodine, but its effective dilution surpasses that of iodine in blood serum. Chlorothymol, while moderately active in the presence of water and broth, fails to prevent growth in a saturated solution under the conditions imposed by the other tests. Thymol,

TABLE I  
COMPARATIVE STUDY  
ORGANISM: *MONILIA TROPICALIS*. TIME: 15 MINUTES

DRUG	WATER SUSPENSION (EFFECTIVE DILUTION)	BROTH SUSPENSION (EFFECTIVE DILUTION)	HIDE DUST SUSPENSION (EFFECTIVE DILUTION)	VESICLE FLUID SUSPENSION (EFFECTIVE DILUTION)	BLOOD SERUM SUSPENSION (EFFECTIVE DILUTION)
Iodine	1:75,000	1:20,000	1:5,000	1:2,000	1:1,000
N-hexyl resorcinol	1:20,000	1:15,000	1:5,000	1:2,000	1:2,000
Chlorothymol	1:9,000	1:8,000	None*	None	None
Thymol	1:2,000	1:2,000	1:1,250	None	None
Sodium hypochlorite	1:7,500	1:2,500	1:750	1:500	1:500
Salicylic acid	1:1,250	1:1,100	None	None	None
Benzoic acid	None*	None	None	None	None
Sodium thiosulphate	None	None*	None	None	None

\*"None" indicates that even a saturated aqueous solution was not strong enough to kill the organisms.

Approximate dilution representing saturation:

Chlorothymol, 1:5,000

Thymol, 1:1,100

Salicylic acid, 1:500

Benzoic acid, 1:350

Sodium thiosulphate, 1:2

the third member of the series chosen for these modified tests, while outranked by the preceding, retains a power to kill in the presence of hide powder, but loses this power, even in a saturated solution, in the presence of vesicle fluid and blood serum.

Of the four commonly used drugs, presumably salicylic acid and benzoic acid enjoy a popularity of greatest duration. Under the conditions imposed, salicylic acid appears to act only in high concentration in the presence of water and broth suspensions, and fails entirely even in saturated solution in the presence of the other three substances used. Even more striking is the complete inability of benzoic acid to kill in saturated solution under any of the conditions imposed by these experiments. The same is to be said of sodium thiosulphate, though its solubility is much greater. Apparently, so far as these tests are indicative, the recommendations of Osborne<sup>3</sup> regarding sodium hypochlorite are well founded. While a greater concentration is necessary, it retains an ability to kill throughout the series of tests.

Results obtained under the conditions of these experiments reveal that fungicidal tests made in water and broth suspensions may be valuable for making comparisons between various drugs in the laboratory but can give only a rough idea as to the manner in which they will react in actual clinical trial.

The need for a standardized method of testing in the presence of proteins is indicated. The authors have used egg white as an easily obtained protein material for testing, but attempts to standardize its effect against that of blood

lacquer.\* This is illustrated in Fig. 1, while in Fig. 2 the method of application of the lacquer to the horizontal film is demonstrated.

Originally in this work an attempt was made to use a collodion film, but this was found to distort the red blood cells so that it was abandoned. The

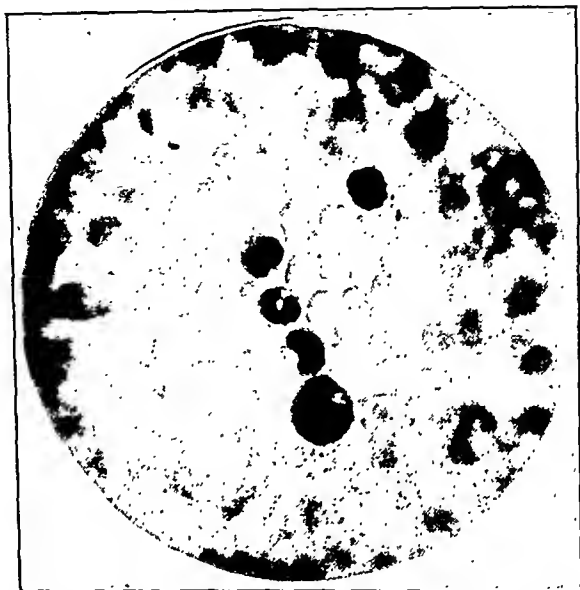


Fig. 3.—Photomicrograph of a blood smear of chronic myeloid leucemia with a 2 mm. apochromatic objective and oil immersion. Wright's stain and preserved under lacquer for three months.

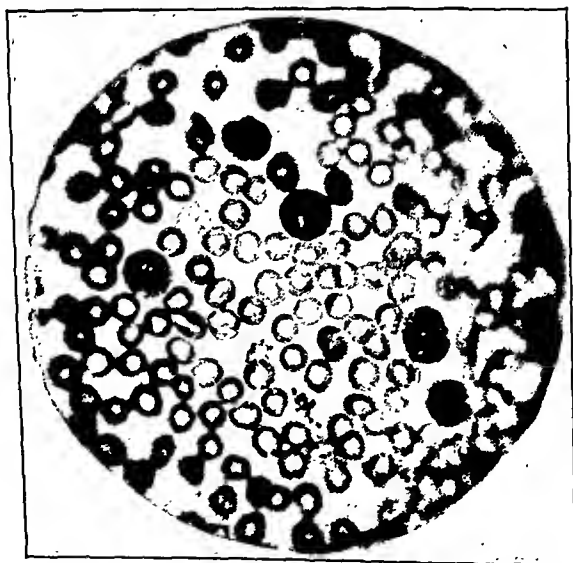


Fig. 4.—Photomicrograph of a blood smear of chronic myeloid leucemia with a 2 mm. apochromatic objective and oil immersion. Wright's stain and preserved with balsam and a cover glass for three months.

lacquer has been found to preserve the original appearance of the red blood cells with but slight distortion. Even with damp weather the lacquer dries

\*The glass jar is stocked by any photographic supply company and is manufactured by a New York specialty firm.

# LABORATORY METHODS

## THE PRESERVATION OF BLOOD FILMS\*

THOMAS K. RATHMELL, M.D., AND HAROLD W. JONES, M.D., PHILADELPHIA, PA.

THE preparation and preservation of blood films for microscopic study is important. Canada balsam plus a suitable cover glass has been the approved method. Slides thus preserved are usually commendable from the microscopist's viewpoint, providing the original staining technic is good. The handling of balsam, cover glasses, and the period required for drying the mounted film is time consuming.

### NEW METHOD

We have substituted a technic which will enable one to quickly render a film permanent after staining. It is cheaper and the time required for the permanent preservation of one or more slides is a matter of a few seconds.

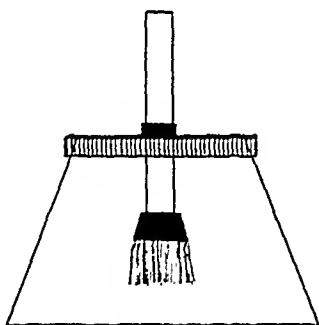


Fig. 1.

Fig. 1.—Commercial glass jar with Sable brush soldered in place. This is an air-tight receptacle for lacquer.

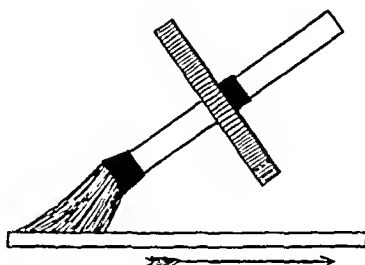


Fig. 2.

Fig. 2.—Method of coating the blood film.

*Technic.*—This consists of the application of a thin coating of lacquer† by means of a Sable brush‡ to the surface of a clean, dry, prepared blood film which has been stained. If the lacquer on prolonged standing becomes cloudy, its transparency is readily restored by the addition of a small quantity of transparentizer. This is always available from the lacquer supply firm.

*Apparatus.*—A commercial glass jar with an air-tight cover in which the brush may be mounted permanently is recommended as a container for the

\*From the Department of Medicine, Jefferson Medical College.

Received for publication, August 16, 1934.

Prepared under grant from Mead Johnson Company.

†The lacquer is a commercial product of the J. H. Weil Company, 1315 Cherry Street, Philadelphia. (Lacquer Number 14.)

‡The Sable Brush, Number 16, may be obtained from any art supply company.

the blade and the holder to keep the long bevel in contact with the cutting edge of the old regular microtome knife. The Sextoblade must have the back snapped off and the Durham Duplex blade works better if it is broken in two. These blades are easily broken off by clamping them in a vise that holds the entire length of the blade.

We have not found a cement that would not hold even though we have used water soluble glue. We have had most experience with Dueco Household Cement and find it satisfactory. The blades are easily removed and the

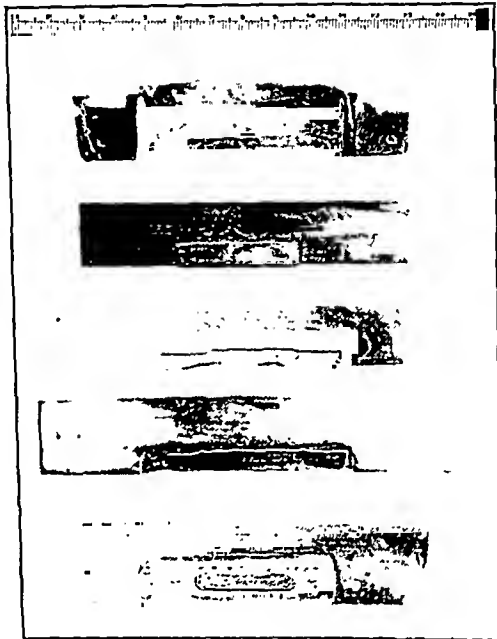


Fig. 1.—1, A regular microtome knife with the cemented blade projecting too much. 2, A piece of sheet iron (gauge 16) with a hollow bevel. This sheet iron was hardly rigid enough when tested out on a block of dense leiomyoma about three-fourths inch square but worked on softer tissues. 3, A piece of monel metal machined into the shape of a regular knife and an exaggerated hollow grinding. 4, A piece of monel metal (same as No. 3) with a Schick blade cemented on the flat surface. This is our favorite set-up for small sections. 5, A piece of babbit poured in a crude mold and dressed into shape with a rasp and emery paper. The babbit does not warm a frozen block or paraffin block as fast as steel or monel metal.

cement stripped off with an old discarded blade. No solvent is necessary but acetone may be used. Be sure to wipe off the oil or grease on new razor blades before gluing them.

A couple of horseshoe magnets are convenient for holding the blades together while the cement is setting.

The knife holders on some freezing microtomes are not adjustable and the resulting bevel is not always satisfactory. This may be compensated for in making the razor blade holder, or by the use of lugs, or by set screws properly added to the knife holder.

The illustration shows some makeshifts we have used with satisfactory results.

Mr. P. M. Schreck, Junior Medical Student at Baylor University School of Medicine at Dallas, Texas, assisted in this work.

within seventy-five seconds after application. It is not affected by either xylol or cedar oil when dry. It probably has a refractive index approximately that of glass which is usually 1.5.

Observations of slides permanently preserved with laequer were made with a Zeiss binocular microscope, utilizing 10X oculars and a 20 mm. dry objective or a 2 mm. apochromatic oil immersion objective. Under the dry objective the film has an appearance superior to that of the unmounted slide. The nuclei of the cells are particularly distinct. With the oil immersion objective the film is equal in refractiveness to one mounted in balsam and a cover glass. Freyfeld's granules and Doehle's inclusion bodies, as well as other cytoplasmic or nuclear structures, are well detailed.

Those who may wish to use this method are cautioned to have a dry slide and to apply the laequer with one stroke of the brush. It is desirable to obtain a thin smooth application of the laequer. This need not be performed with excessive rapidity, but the brush should not carry an excess of the laequer. Wiping the brush on the rim of the glass jar before touching the slide is recommended.

We wish to thank Dr. Frederick Kramer and Mr. Joseph Poppel for their aid in the preparation of the illustrated apparatus.

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## THE USE OF RAZOR BLADES FOR TISSUE SECTIONING\*

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I. A. NELSON, M.D., TULSA, OKLA.

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**R**AZOR blades may be used in sectioning tissues with either freezing or paraffin microtomes without the use of special razor blade clamp holders. To use a razor blade one merely needs an old discarded regular microtome knife, or a piece of metal sharp on one edge (similar to a microtome knife) and some cement. This laminated assembly seems to diminish quivering.

Good support of the cutting edge is essential to success in using razor blades. With a heavy holding knife, or its equivalent, which has a sufficient hollow grinding, blades that have a long bevel may be so placed that the hollow sharpened edge of the holding knife makes intimate contact with the long bevel very close to the edge or the second short cutting bevel. Some blades have two bevels.

We have used the Schick razor blade for over five years in a modified Spencer razor blade clamp holder, and found it the best in quality of steel, in bevel, and in rigidity for frozen and paraffin sections. Recently we wanted to cut blocked tissues by frozen method but our blocks were too large for the effective area covered by a Schick blade in a Spencer freezing microtome. We found that either a Sextoblade blade or a Durham Duplex blade could be used by gluing to an old knife if we provided support for the bevel. This support can be obtained by using sufficient glue to act as a filler in the space between

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\*From the Pathology Laboratory of St. John's Hospital.  
Received for publication, September 16, 1934.

collected quantitatively over long periods. They anastomosed the common duct to the right ureter after removing the right kidney. Although the original purpose of the technic was defeated because of the impossibility of separating chemical substances common both to urine and to bile, they published the method because of its suitability for other investigations.

Kapsinow, Engle and Harvey<sup>8</sup> described an intraabdominal biliary exclusion from the intestines through a cholecystnephrostomy. Since Pearce and Eisenbrey first introduced their technic for intraabdominal drainage, the biliary channels have been transplanted to many parts of the intestinal tract and urinary system.

The need for an exact means of bile collection is indicated by the fact that major problems in connection with the bile output which were first formulated nearly three generations ago are still unsettled.

The physiology of the bile and its relation to the various metabolic functions of the body has recently acquired renewed interest because of the work of Hooper and Whipple, Rous and McMaster, and others. In attempting to study the important question whether or not bile is essential to life, the physiologist has been confronted with the difficulty of finding some method which would not entail excessive inconvenience to the animal or to the investigator.

The ability of the white rat to adapt itself to almost any reasonable dietary regimen, and the economy of its maintenance, have resulted in its being the animal of choice in most physiologic laboratories. The rôle of bile in nutrition and metabolism has been almost universally accepted as important. Hence no such studies are complete without considering the bile factor. That the rat is without a gallbladder makes the interest of such studies even greater. For these reasons it is believed that there is need for a bile fistula technic which will meet the following requirements:

1. Bile must be completely eliminated from the animal so there remains no possibility of absorption or ingestion by mouth.
2. Collection at varying intervals must be carried out with ease.
3. The bile must be collected quantitatively and uncontaminated.
4. The animal should suffer a minimum of discomfort.

So far as is known, no technic for a bile fistula exists which meets all these requirements, except that described herein. The latter is the outcome of prolonged experimenting with internal and external reservoirs of rubber and glass, of many sizes and shapes, variously placed in the animal. It is believed that this technic meets most, if not all, of the requirements for a satisfactory bile fistula in the white rat.

The complete assembly of the collecting apparatus for carrying out the bile fistula operation is shown in Fig. 1. The glass bulb, *A*, serves as a bile reservoir. Its capacity may be varied according to the animal used. For animals weighing approximately 200 gm., a 6 c.c. bulb is employed. It has two nipples at the top, *B* and *E*, one placed vertically for withdrawal of bile, the other horizontally to admit bile. The nipple *B*, admits a 19 gauge needle and holds firmly in position a flexible rubber tube, *C*, 3 mm. inside diameter and approximately 4 cm. in



# TECHNIC FOR A BILE FISTULA IN THE RAT AND DEMONSTRATION OF THE INDISPENSABILITY OF THE BILE\*

LESTER SAWYER, M.A., AND SAMUEL LEPKOVSKY, PH.D., BERKELEY, CALIF.

MANY investigators have devised methods for studying bile. Each method has had an inherent difficulty which has led to other technics for excluding bile from the intestines. The dog has been the animal of choice in most bile fistula investigations recorded in the literature.

Of the many methods described for making biliary fistulas, one of the following procedures has been generally employed:

1. Suturing the gallbladder to the abdominal wall and opening it so that it drains to the exterior.
2. Suturing a cannula or rubber tube in the gallbladder.
3. Excising the common bile duct at the point of entrance to the duodenum, together with a piece of duodenal wall, and suturing the latter to the skin.
4. Placing a cannula in the common bile duct.

Bidder and Schmidt<sup>1</sup> in 1852 published their classical observations on the composition of fistula bile obtained from a simple external biliary fistula. Severe infections were produced, and the animals usually died within a very short period. The animals were able to liek themselves unless they were strapped all the time.

Dastre<sup>2</sup> collected bile from a fistula in a bag suspended from the neck of the animal. But, as Hooper and Whipple<sup>3</sup> point out with regard to similar findings in human beings with bile fistulas, there is no certainty that some bile did not reach the intestine, since autopsy findings are not available, and since in the light of our knowledge, new openings into the intestine may occur.

Pawlow transplanted to the skin a flap of duodenum containing the ampulla of Vater. Mann<sup>7</sup> described a method of transplanting a portion of the duodenum containing the common duct through an abdominal incision into the subcutaneous layers, the duct later being connected with the body surface.

Rous and McMaster<sup>4</sup> utilized a long tube attached to a cannula inserted into the common duct. The cystic duct was ligated and the bile collected in a sterile balloon.

Pearce and Eisenbrey<sup>5</sup> have suggested a different approach to the problem. In connection with studies on the relationship of the spleen to blood destruction, they found it necessary to develop some method by which bile might be

\*From the Department of Anatomy and the Institute of Experimental Biology, University of California.

Received for publication, October 26, 1934.

Aided by grants from the Rockefeller Foundation of New York City, and the Board of Research and the College of Agriculture of the University of California.

inserted into the duct. The probe is removed from the cannula, and a second ligature made taut in the groove. Bile can now be observed to flow from the cannula. The entire duodenum is returned to the abdominal cavity, the ends of the ligatures remaining outside.

The rubber tubing *C* of the assembled bulb is slipped over the special needle shown in Fig. 1. The needle is passed through a point just lateral to the right sacrospinalis muscle about midway between the last rib and the innominate bone. The rubber tubing is next drawn to the dorsal surface, and the bulb is introduced into the abdomen. With forceps, the rubber tubing *F*, adjusted to a satisfactory length, can be securely slipped onto the cannula. The ends of the ligatures may here be utilized advantageously. The ends of the first ligature are used to secure the tubing on the cannula, and to retain both in line with the duct on the duodenum. The threads are cut close to their knots, and the duodenum is replaced in its normal position. The abdomen is closed in the usual manner, and the animal placed on its left side. The special needle is drawn through the short length of rubber tubing *D*, which is adjusted to a satisfactory position on tube *C*. Removal of the special needle completes the operation.

The exposed rubber parts are seldom destroyed by the animal. Should a rat be encountered which persists in chewing the tubing, a snugly fitting glass or metal tube may be placed over the rubber. This is seldom necessary if tame animals are used and kept in individual cages.

The tube *C* serves a dual purpose. It allows air to be expelled from the reservoir as bile flows in, and admits a blunt 7 cm. 19 gauge needle attached to a syringe of appropriate capacity for the withdrawal of bile. Merthiolate is used in the bulb as a preservative. The reservoir may be rapidly and completely emptied of bile with a minimum of discomfort to the animal.

In a series of experiments carried out to test the technic, female rats approximately five months of age were used. Females were given preference because of the additional information of the state of the animal afforded by following the estrous cycle. The animals occupied metabolism cages in a constant temperature room, and were fed a standard stock diet designated Diet I.\* Food, water, urine, bile, and animal weights were measured twice daily, at 7 A.M. and 7 P.M.

Three groups of animals were designed to act as controls for the technic. Group I consisted of twenty animals, the bile ducts of which were ligated. Group II consisted of twenty animals which carried the entire collection apparatus; the bile duct was cannulated but a plug placed in the rubber tubing *C*, so that bile was unable to flow into the collecting bulb because of the air pressure encountered therein. Group III consisted of eleven animals which carried the collection apparatus, but the bile duct was not cannulated, a plug being placed in the tubing which ordinarily receives the cannula.

No differences were found between the reactions of Groups I and II. The length of life of these animals and their general downhill course closely ap-

\*Diet I: Whole wheat, 67.5; casein, 15.0; whole milk powder, 10; sodium chloride, 1.0; calcium carbonate, 1.5; and milk fat, 5.0.

length. A short length of pressure tubing, *D*, fits tightly over *C*, to retain it on the outside of the animal. Attached to the nipple *E* is a piece of thin-walled, very flexible, 1 mm. rubber tubing, the length of which is fixed at operation. The cannula *G*, which is inserted into the bile duct, is made of 18 or 19 gauge stainless steel or silver hypodermic needle shaft tubing; its length is approximately 8 mm. There is a gentle taper from the center to the end which inserts in the bile duct. The duct is held in position by a fine silk ligature which fits into a narrow groove circumscribing the cannula about its center.

The operation is carried out with the animal under ether anesthesia. The anesthetic is efficiently administered through the use of the apparatus described by me,<sup>9</sup> substituting a mask for the tracheal cannula. Aseptic surgical technique is employed throughout all operative procedures.

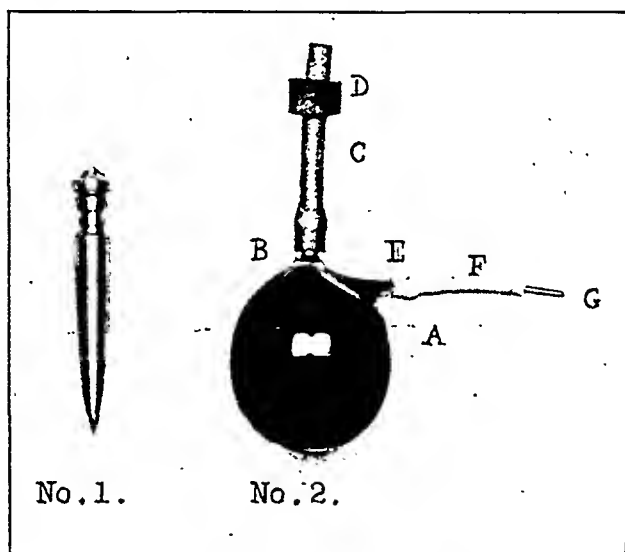


Fig. 1.—1, Special needle. 2, Collecting apparatus (assembled).

The animal being properly prepared for operation, an incision is made in the right upper quadrant just lateral to the right rectus muscle, approximating in length the diameter of the bulb to be used. The duodenum usually comes into full view, and is pulled gently through the incision. The bile duct is seen coursing through the mesentery, and along the anterior wall of the duodenum for some distance. It is this section of the duct which is cannulated because it is firmly attached, its walls are strong, and the size of the lumen is greatest.

With a fine needle, a long silk ligature is placed beneath the duct close to its opening into the duodenum, and securely tied. The duct becomes filled with bile a few minutes following ligation. A second ligature is passed beneath the duct close to the first, avoiding certain vessels which are sometimes found coursing over the duct. With an iridectomy scissors, and using the loose ends of the first ligature as an anchor, the duct is hemisected; a blunt probe is introduced. The probe is withdrawn and inserted loosely into the untapered end of the cannula. Using the probe as a convenient handle, the cannula can be easily

not caused by the insults sustained during the operative procedure, nor by the presence of the collecting apparatus within the body cavity.

The lack of appetite, the tremendous loss of weight, and the rapid downhill course to death, again focus attention on the importance of bile in the life of the animal. It is believed that the fistula described will prove a valuable method of investigating further the rôle of bile in the life of the rat, an animal almost universally used for experimental purposes, whose biliary system differs from that of most vertebrates in that the gallbladder is entirely absent and without a homologue.

The writers wish to acknowledge with thanks the aid and guidance given to this work by Professor I. Maclaren Thompson, of the Anatomy Department of this university.

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proximated each other. At autopsy, some fifteen to thirty-five days following operation, the animals of Groups I and II were found to have bile ducts greatly distended with bile, and livers presenting the usual destruction encountered in biliary obstruction.

The animals of Group III were practically unaffected by the operation. No change occurred in food or water consumption, or in urine output. These elements manifested the diurnal variation shown prior to operation. One hundred and twenty days postoperative, the animals had maintained or gained weight, and had continued to run normal eyes. A few of these animals were sacrificed after thirty days, and at autopsy were found to present little or no change attributable to the presence of the collecting apparatus.

A fourth group of animals constituted the experimental group, and upon them the complete technic was carried out so that the total output of bile drained into the reservoir. These animals recovered from the operation and appeared normal for from twelve to twenty-four hours. The next period was usually accompanied by a gradual decrease in activity, weakness, coma, and finally death. The food intake dropped to almost zero. The water intake was in most cases lowered, and the volume of urine considerably reduced. Some animals survived up to ninety hours. During the interval between operation and death, a tremendous loss of weight was noticed, far more than would be lost in a normal starving animal. Table I illustrates data which exemplify the results obtained in 6 animals over a period of forty-eight hours after the operation (with the exception of animal GH3640, which was observed for twenty-four hours).

TABLE I  
THE DIURNAL VARIATIONS OF THE SECRETION OF BILE IN RATS

ANIMAL	WEIGHT AT OPERA- TION GM.	WEIGHT AT DEATH GM.	TOTAL LOSS IN WEIGHT GM.		BILE		SECRETION	
					7 A.M. C.C.	7 P.M. C.C.	TOTAL FOR 24 HOURS C.C.	G.C. PER KILO PER 24 HOURS
W3672	236	202	34	1st 24 hr.	4.4	6.8	11.2	50.4
				2nd 24 hr.	4.9	4.6	9.5	42.7
W3306	221	187	34	1st 24 hr.	4.8	6.5	11.3	55.3
				2nd 24 hr.	4.9	4.3	9.2	45.0
BH3436	211	178	33	1st 24 hr.	5.4	6.0	11.4	58.1
				2nd 24 hr.	4.9	4.9	9.8	49.9
W3318	240	200	40	1st 24 hr.	5.0	8.2	13.2	59.4
				2nd 24 hr.	5.1	7.3	12.4	55.8
BH3433	209	178	31	1st 24 hr.	3.9	5.7	9.6	48.9
				2nd 24 hr.	5.7	5.4	11.1	56.6
GH3640	249	220	29	1st 24 hr.	4.9	5.6	10.5	44.1

If at any time in the life of the animals in Group II the plugs were removed from tube C, allowing drainage of bile into the reservoir, they reacted exactly like the animals of the experimental group. If the plug were replaced before the animal lost appreciable quantities of bile, the animal would survive and continue in the same manner as the others of this group.

It is believed that the groups of animals acting as controls for the technic prove conclusively that the death of the animals in the experimental group was

duction to my method has kindly informed me of the fact that egg methods of blocking tissues antedate the paraffin methods; that Neuman used egg albumen for embedding the vitreous body in 1862; and that many others have worked with egg proteins. Some of the old methods were similar to the one I developed in trying to imitate with oxalated blood that which may be seen in many inflammatory and pathologic tissues.

If one anticipates impregnating protein-blocked tissues with paraffin for very thin sections, our preliminary experiments indicate that the whole egg complex is possibly the best protein because the lipoids do not interfere with freezing methods or staining. With a *slow* method of paraffin impregnation, the extraction of the lipoids leaves a fine delicate reticulum into which the paraffin can permeate. Possibly better suspensoid substances can be developed and added to protein emulsions, especially the blood proteins. But the lipoids are already in such a highly disperse state in fresh eggs and are extracted readily by the dehydrating agents used in paraffin embedding that improvement seems difficult. A smooth emulsoid formalin fixed protein is rather resistant to paraffin impregnation.

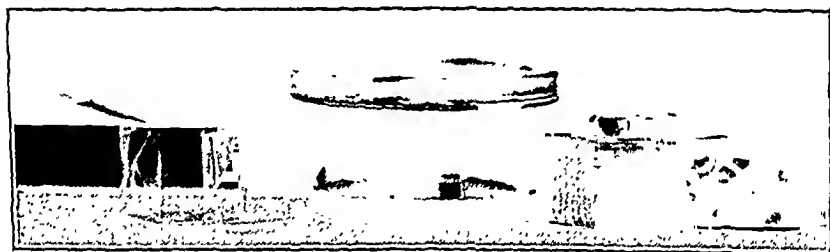


Fig. 1.

Probably the most available commercial protein is egg white powder. This can be bought in candy factories cheaper than most other dehydrated proteins. Three grams of egg white powder in 10 c.c. of water, or one gram of egg white powder in 5 c.c. of blood serum, left over from serologic tests, has been found satisfactory.

We have not found any bacteriostatic agents up to the present time which can be added in sufficient quantity to the high protein content emulsions used. They alter the emulsoid state or interfere with staining. Prepared emulsoids can be kept in the refrigerator or be made fresh from the powdered protein in a few minutes. The search for a powdered protein was prompted by this difficulty in finding a bacteriostatic agent. We forgot to return to the refrigerator some laked oxalated blood and the odor of putrefaction which developed convinced us that those attempting this method should be warned and given some alternate procedure if they did not have good refrigeration. The age also of some emulsoids seems to affect their water binding power during fixation so that the blocks may not have as much volume as with fresh emulsions. But as long as small batches are made, they should not age sufficiently to affect their utility and accidental putrefaction then becomes a mere incident.

## THE USE OF PROTEINS FOR EMBEDDING SMALL TISSUES FOR SECTIONING BY THE FREEZING METHOD\*

I. A. NELSON, M.D., TULSA, OKLA.

THE summer of 1934 was so hot in this section of the country that paraffin cutting was quite a problem. As frozen technics are becoming quite generally adopted, the following method was developed instead of some better paraffin complex. This is not an impregnating method in its present form and cannot be used on tissues which do not hold together within themselves. However, nearly all the actual routine surgical bits of tissue can be blocked and sectioned by this method. It is economical and rapid.

The rationale of this method came as a result of speculations and reading about hyaline thrombi<sup>1</sup> and fixed colloids of various sorts in lymphatics, vessels, and open spaces. In routine tissues one often sees that formalin fixed proteins hold leucocytes and bits of tissue even though the cut section is passed through numerous solutions.

This method takes advantage of the fact that emulsoid colloids may be chosen which wet and stick to the tissues and which do not pass through membranes that are traversed by many coagulating substances. The advantage of using dialysis instead of adding the coagulant or fixative to the emulsoid is that the emulsoid has greater freedom to satisfy its particular water requirement and still not precipitate in disconnected clumps.

The emulsoid colloids which we have found the most satisfactory from the standpoints of good blocking properties, availability, and economy, are the blood and egg proteins. Different proteins require varying percentage concentrations, but whole egg and laked oxalated whole blood already contain enough protein.

Oxalated blood which is left over from clinical tests and which contains over 14 gm. of hemoglobin per 100 c.c. has been found convenient and satisfactory. Other modifications included in this paper may be used by those who cannot get blood or who feel that the blood used might be confused with blood states in the tissues. A small bit of saponin powder is most convenient for changing the suspensoid cellular hemoglobin into an emulsoid. When the bloods from various groups are pooled, it is possibly better to lake the cells of each blood before adding them to the pool. The oxalated blood may be allowed to stand until the red blood cells have settled, the plasma decanted into one container, the cells laked and added to a separate pool. Equal parts of each may then be mixed for uniformity.

Whole raw eggs make satisfactory tissue blocks. The yolk is broken and stirred into the albumen. Dr. J. M. Thurninger who recently had a brief intro-

\*From the Pathology Department and The Mark Finston Research Fund of St. John's Hospital.

Received for publication, October 29, 1934.

This method was presented to the Society of Medical Sciences of the Oklahoma University School of Medicine on October 18, 1934.

of the end of the wooden block and fold the cellophane over this. There is usually some wrinkling of the protein block. The paper keeps the bits of tissue in one plane. Thus one is assured of cutting and looking at all the bits of tissue submitted. The boxes should be as small as necessary to produce thin sections with the particular freezing microtome being used. The two projecting folds of cellophane are convenient when handling the box.

The coagulant used is formalin solutions from 20 to 40 per cent in strength. It does not interfere with freezing methods, and penetrates rapidly.

The steps in preparing tissue blocks may be summarized as follows:

1. Place the cellophane box in a small dish (we use low Stender dishes).
2. Place the bits of tissue in the box (previous fixation is optional).
3. Pour the emulsoid protein over the pieces and stir or orient them. Air bubbles of significant size should not be trapped at the interfaces.
4. Pour about 5 c.c. of 20 to 40 per cent formalin into the dish and around the cellophane box. The level of the formalin should be a little below the level of the emulsoid. It should be neutral or slightly alkaline so as to preserve the proteins about their isoelectric points. The dish may be covered to keep down the formaldehyde.
5. Allow from two to four hours for fixation. This depends on the amount of emulsoid used, the strength of formalin and the temperature of the room.
6. Peel off the cellophane. This is most conveniently done at the sink, allowing running water to wash off the strong formalin.
7. Trim the block and proceed as with any formalin fixed tissue.

The satisfactory reports that have come in to me from workers to whom the above outline has been submitted confirm our experience and seem to justify publication at this stage.

Dr. H. Nauheim (Pathologist at Morningside Hospital, Tulsa, Oklahoma) informs me he has blocked bits of tissue removed by the needle puncture biopsy method with good success.

Other objects besides tissue such as parasites and fecal concentrates containing parasite ova may be blocked and sectioned for mounting.

Those who happen to use fresh oxalated blood will discover intact leucocytes in the blocking media. This observation led to studies on bone marrow blood and this preliminary report must be included at this time. Dr. E. E. Osgood demonstrated, at the American Medical Association Convention at Cleveland, Ohio (1934), a method of drawing off bone marrow from the sternum with a needle, oxalating it and making smears in the same manner that smears are made from oxalated whole blood. The oxalated bone marrow blood may be laked and fixed by this method. In thin sections the cells may then be studied in their spherical shapes by the usual histologic methods. If the patient is anemic, the bone marrow blood proteins may be enriched with some stock laked red cell hemoglobin emulsion. We have had no leukemia cases to try this method on, but anticipate no contraindication.

#### SUMMARY

A simple method is described in which emulsoid proteins are used for blocking routine surgical bits of tissue.

Special consideration is given to freezing and routine staining techniques.

The principle of dialysis is utilized and the reasons for its use are given.



Several other commercial powdered proteins have been tried, such as: caseo (casein) glue; monite (casein) glue; caseo protein milk; dehydrated ox blood hemoglobin; dehydrated beef muscle; and dried buttermilk, sold for chicken feed. Each of these has some property that keeps it from being recommended.

Gelatin methods have been described<sup>2</sup> and are used to some extent. A high percentage of gelatin can be dissolved in blood serum and remain liquid at room temperature. But the expense is greater and the sections seem to shear

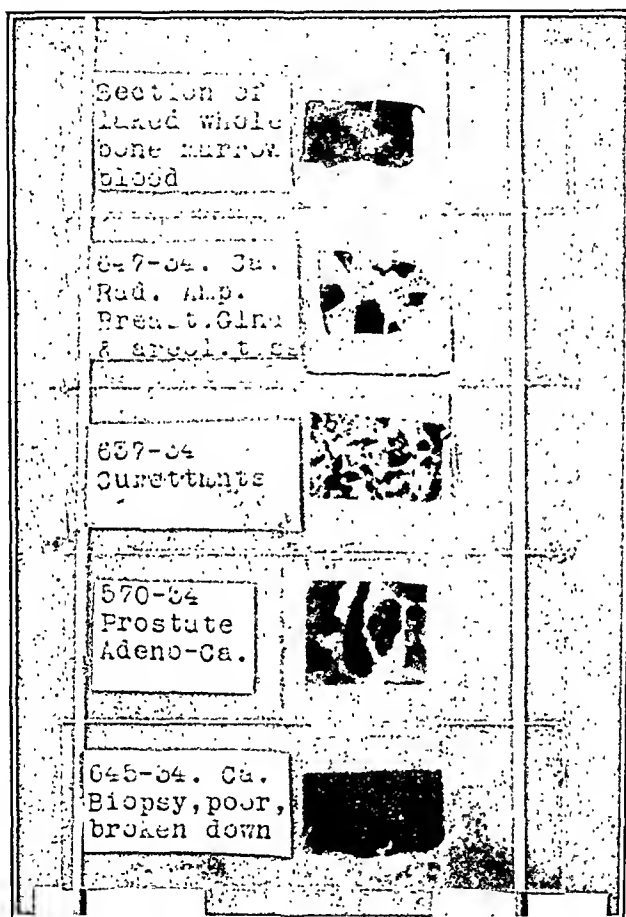


Fig. 2.

and crack. The use of plasma seems to improve the sections but the necessity of buffering the high calcium content of commercial gelatins keeps this from being recommended.

The dialyzing membrane is made by folding a piece of ordinary wrapping cellophane (not waterproof) measuring about 2 by 4 inches into the shape of a cubical box in the same manner that paper boxes are made for paraffin embedding. The cellophane is folded over a wooden block with square ends measuring about three-fourths of an inch square so that the ultimate block will be covered by a glass coverslip seven-eighths of an inch square. If there are only a few very small bits of tissue, cut out a piece of heavy blotting paper the size

The accompanying figure shows the relation of static deflection to vibrations per minute for 4.7 and 10 cm. wide rubber bands. It appears that rubber does not follow the above equation perfectly for the wider band requires greater static deflection to attain the same period.

Eescape from vibration is best accomplished by making the period of the suspended mass twice the period of the vibrations of the building. Since it was desired to escape vibrations of a frequency of eighty or more, the curve for the

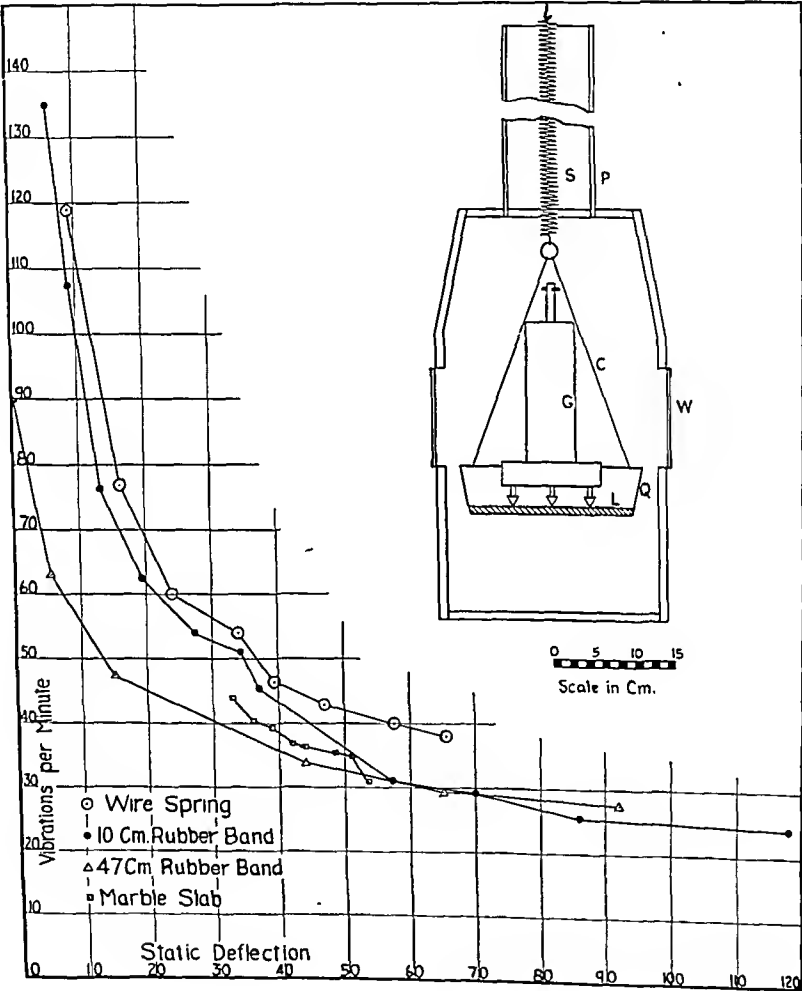


Fig. 1.

period of the suspended marble slab shows the accomplishment of this aim. It was found, however, that the frame of the building changed its position slightly with the wind and this made it necessary to adopt a one point suspension method.

Rubber has the advantage over springs in that it has no resonance, but springs follow the above equation more perfectly. However, for the single suspension method a spring of No. 12 coppered Bessimer steel with 300 turns of  $\frac{3}{8}$  inch radius was used. The static deflection of the spring required to give the proper period is shown in the figure. When thus stretched the spring was 203 cm. long.

Cellophane is used for the first time so far as we can determine. It simplifies the application of dialysis.

The use of laked whole oxalated blood is used for the first time so far as we can determine.

The possibility of studying leucocytes and bone marrow cells in the blood and in their spherical shapes is suggested.

Mr. P. M. Sehreek, junior medical student at Baylor University School of Medicine, Dallas, Texas, kindly assisted with this problem during his summer vacation.

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Mallory and Wright: Pathological Technique, ed. 7, W. B. Saunders Company, p. 54.  
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### A SUSPENSION DAMPED FOR LOW FREQUENCY VIBRATIONS\*

J. PERCY BAUMBERGER, D.Sc., AND STEPHANIE SIGURDSON, A.M.,  
STANFORD UNIVERSITY, CALIF.

IN CONNECTION with some work in a third floor laboratory, it was found impossible to use a sensitive galvanometer because of the vibrations continually occurring. We are deeply indebted to Dr. L. S. Jacobsen, of the Mechanical Engineering Department, for suggesting methods by which these difficulties could be surmounted. The most difficult part of the situation was the relatively low frequency of the vibrations, which were eighty-five per minute and were due to walking. Since such a situation very commonly arises, our experience in its solution may be of sufficient interest to justify the detail given in this note.

With the intention of having the galvanometer, source of light, and other parts of the apparatus, all suspended, a seventy-five pound slab of marble, 57 by 83 by 4 cm., was hung from the ceiling by four straps cut from automobile tire inner tubes. The part of the room where the apparatus was to be set up had a slight slant on the ceiling, and therefore the bands had to be of different lengths. The relation between the period and the strength or static deflection is given by the equation:

$$N = \sqrt{188 \frac{1}{\Delta St}}$$

where  $N$  = cycles per minute

$\Delta St$  = static deflection in inches

All rubber bands were brought to the same period by loading equally and then trimming down to a width that gave equal frequency.

\*From the Physiology Department of Stanford University.  
Received for publication, November 5, 1934.

the full length of the tube and projects beyond the orifice to end in a loop. Such tubes may be detached from the cage, filled with the pellets, and resuspended in a few moments. The jaws of the tube are so arranged that in the process of withdrawing the pellets the rat agitates the tube to some extent and this, together with the loop of wire, prevents the pellets from clogging and causes them to continue to fall to the lower end of the tube. The rat seems to enjoy this type of feeding. He immediately seizes the pellets in his jaws and pressing with his forefeet on the tube exerts considerable strength, and thus succeeds in pulling

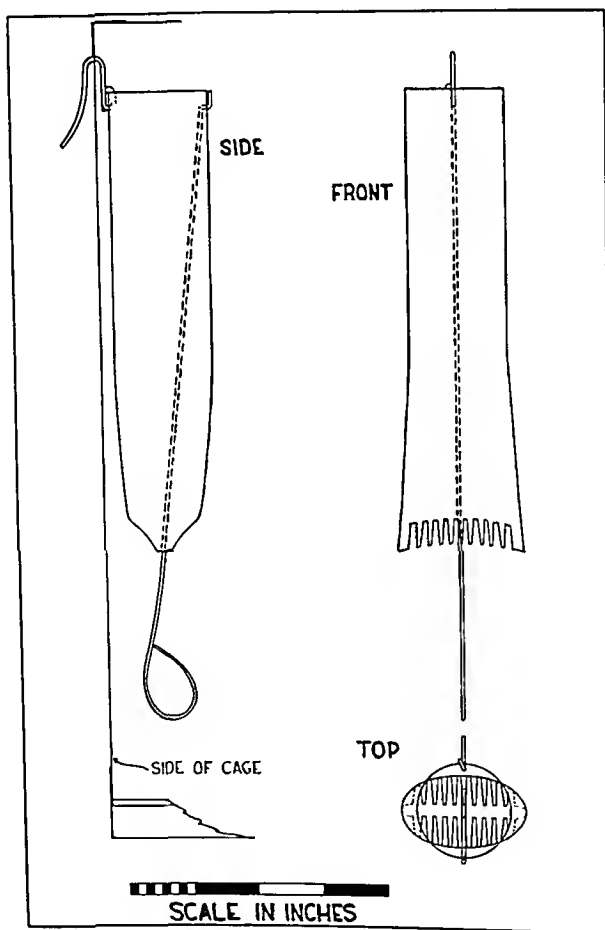


Fig. 1.

the pellets out in spite of the tight set of the jaws. Usually when the tubes are refilled, several rats will attempt to obtain pellets at the same time, crowding each other away from the tube. This jostling seldom leads to a fight but is all in good nature. When a pellet is obtained, the rat retires to the other side of the cage and remains there sitting on his haunches very much like a squirrel completely. Occasionally rats develop the habit of emptying the feeding tube with a nut. They store the food in one corner of the cage, but in all cases, the food thus stored during the course of the day is eaten. A tube of the size indicated contains food for six rats for a day.

To support the galvanometer (*G*), the handle was removed from a 20 cm. cast-iron frying pan (*Q*) and three holes were bored into the sides at points 120° apart, from which were run chains (*C*) to the end of the spring (*S*). The weight required to give the correct period was 11.15 kg. and the weight of the galvanometer was 1.85 kg. Melted lead (*L*) was poured into the pan until the pan and lead were brought to the required weight, i.e., 8.30 kg. The lead formed a flat surface on which it was easy to level the galvanometer. To protect the spring from air currents, we put a galvanized sheet-iron pipe (*P*) around it and fastened that to a square box surrounding the pan and galvanometer. Two sides (*W*) of this lower box were of glass which could be slipped in and out making the instrument easy to handle. The galvanometer and the weighted pan gave approximately the desired period, and therefore were not appreciably affected by the vibrations of the building. Thus by application of the equation relating static deflection to period, vibrations of low frequency can be avoided. In our case we succeeded in obtaining a suspension of sufficient steadiness for proper functioning of a quadrant electrometer.

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### A NEW RAT FEEDING DEVICE\*

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J. PERCY BAUMBERGER, D.Sc., AND SHANNON ALLEN, A.B.,  
STANFORD UNIVERSITY, CALIF.

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MOST rat feeds are in the form of powders because of the nature of the ingredients used in nutritional studies, but this form is inconvenient in several respects. In the first place, the powder is blown throughout the animal breeding room. Second, it is impossible to determine how much of the food is wasted by mixing with the sawdust bedding. Third, no containers are effective in preventing contamination of the food.

There are now on the market several adequate rat diets in the form of compressed cylindrical bodies about 5 mm. in diameter and 10 to 20 mm. long. These are quite uniform in diameter as though forced out from an orifice in the process of manufacture. The constituents of the pellets are such as to provide a complete diet. Moreover, the hardness of this type of food provides a grinding surface for the rats which is completely absent in the powder form diets. Rats which are fed the latter compensate by grinding their teeth on available parts of their cages and the copper tubes of their water bottles with a surprising amount of damage resulting to both. The new pellets have a more natural effect on the rats' teeth and eliminate much damage to equipment.

In Fig. 1 is shown an apparatus which enables the rat to obtain compressed food pellets at will, and at the same time prevents contamination and loss of food. The device consists of a brass tube an inch and a half in diameter and seven inches long, suspended by a hook in the upper end, and the lower end somewhat flattened and provided with teeth. A stiff wire is suspended loosely down

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\*From the Department of Physiology, Stanford University.  
Received for publication, November 15, 1934.

with 1 c.c. portions of concentrated sulphuric acid for periods varying from one minute to seventeen hours. Duplicate analyses were always made. The conversion was complete in two hours, and further increase of time did not alter the value.

TABLE I

CONVERSION OF CREATINE TO CREATININE BY CONCENTRATED SULPHURIC ACID

SAMPLE	CONTENTS	TIME	CREATININE RECOVERED	RECOVERED
			MG.	%
1	1 c.c. creatine solution*	1 min.	0.00	0
2	1 c.c. creatine solution*	10 min.	0.10	10
3	1 c.c. creatine solution*	1 hour	0.70	70
4	1 c.c. creatine solution*	2 hours	1.00	100
5	1 c.c. creatine solution*	5 hours	1.00	100
6	1 c.c. creatine solution*	8 hours	0.96	96
7	1 c.c. creatine solution*	17 hours	1.00	100

\*1 c.c. of creatine solution equals 1 mg. of creatinine.

In Table II are shown values for creatine in urine which contained much pigment. The small amount of color that developed from the action of concentrated sulphuric acid on the urine pigments did not affect the values for creatine. As a further test of the color, 1 c.c. portions of urine were allowed to stand with sulphuric acid for periods ranging up to eight hours. Sodium hydroxide was then added, and the solutions were diluted to 100 c.c. and compared in the colorimeter with 1 c.c. portions of urine which were made alkaline and were diluted directly to the same volume. No difference in the fields could be observed.

TABLE II\*

ANALYSIS OF A HIGHLY PIGMENTED URINE FOR CREATINE BY USE OF SULPHURIC ACID

SAMPLE	CONTENTS	TIME	TOTAL CREATININE
			MG.
1	1 c.c. urine	1 min.	2.09
2	1 c.c. urine	3 min.	2.02
3	1 c.c. urine	12 min.	2.07
4	1 c.c. urine	1 hour	1.96
5	1 c.c. urine	2 hours	2.11
6	1 c.c. urine	3 hours	2.11
7	1 c.c. urine	17 hours	2.11

\*Analysis of the urine for "Preformed Creatinine" by the Folin method gave values ranging from 2.08 to 2.11 mg. per c.c. Analyses for creatine by other procedures gave deep red colors which corresponded to values of from 5 to 10 mg. of creatine per c.c.

In Table III, figures are given to show the influence of an excess of 10 per cent sodium hydroxide on the development of the color. One cubic centimeter samples of the standard creatine solution (1 c.c. = 1 mg. of creatinine) were analyzed by the sulphuric acid method. The amount of 10 per cent sodium hydroxide required to neutralize the acid and to develop the color was 14 c.c. Larger amounts of sodium hydroxide were added to the different samples and each was compared in the colorimeter with a specimen that had received the 14 c.c. of sodium hydroxide. The analyses show that 2 c.c. of sodium hydroxide over the amount needed did not alter appreciably the color which was developed, and that larger amounts decreased the color.

## A METHOD FOR THE DETERMINATION OF CREATINE IN URINE\*

WILSON D. LANGLEY, MAURICE M. ROSENBAUM, AND MYRON G. ROSENBAUM,  
BUFFALO, N. Y.

**D**URING a study of the metabolism of creatine in cases of muscular dystrophy, we compared several methods for the determination of creatine in urine, and with each of them obtained values which were shown to be incorrect. With deeply pigmented urines, the values were often two, and occasionally three times too large. An amount of extra chromogenic material was formed by the action of acid and heat upon some constituent of the urine, and the analysts did not distinguish the color which was due to creatinine from that which was produced by the other chromogenic material. Persons whose eyes were more sensitive to shades of red, and who could match the dissimilar shades in the colorimeter, obtained moderately accurate values for the creatine. Similar difficulty was reported by McCrudden and Sargent,<sup>1,2</sup> and has been experienced by others.

It was found also, that when extra color was absent, the conversion of creatine into creatinine was often incomplete. This inadequacy has been noted by Hahn and Barkhan,<sup>3</sup> who determined creatine of urine by heating the solutions only to 65°, and allowing eighteen to twenty-four hours for the conversion.

We have tested the conversion of urinary creatine into creatinine by concentrated sulphuric acid. Under certain conditions, the conversion was completed in two hours, and a negligible amount of extraneous color was developed. A simple procedure was therefore devised which enabled accurate analyses to be obtained for creatine in urine. Diabetic urines could not be used.

### PROCEDURE

Pipette 1 c.c. of urine into a 100 c.c. volumetric flask. Add, from a pipette, 1 c.c. of concentrated sulphuric acid, mixing after each drop. One or two minutes are required for this addition. Allow the flask to stand at room temperature for two hours. When 2 c.c. of urine are used for the analysis, the flask should stand for five hours.

Into a similar 100 c.c. flask pipette 1 c.c. of standard creatinine solution (1 c.c. = 1 mg. of creatinine), and then add to it 1 c.c. of concentrated sulphuric acid.

To each flask add 20 c.c. of a saturated solution of picric acid (purified if necessary), and then 10 per cent sodium hydroxide so that an excess of 1.5 c.c. over that needed to neutralize the sulphuric acid is present. In our analyses 14 c.c. were used. Precipitation of picric acid or its salt may be ignored. After ten minutes, dilute each solution to the mark, mix, and compare in the colorimeter. The calculations are made as usual.

In Table I, figures to the nearest even percentage are given to illustrate the rate of conversion of creatine into creatinine under the conditions described. One cubic centimeter portions of creatine in distilled water were permitted to stand

\*From the Department of Biochemistry, University of Buffalo Medical School, and the Laboratory of the Children's Hospital.

Received for publication, November 15, 1934.

## ALTERING THE VIM-SCHEFTTEL COLORIMETER FOR GENERAL USE\*

JOHN A. SCHINDLER, M.D., MONROE, WIS.

FOR the small laboratory the expense of a colorimeter is a considerable item. There is on the market a relatively inexpensive instrument, known as the Vim-Schefttel colorimeter, which is of proved reliability and serviceability. As it is at present marketed, however, it is supplied with a syringe calibrated in arbitrary units, so that it is necessary to refer a reading to tables prepared by the manufacturers. At present the colorimeter has been standardized for only a few of the more common procedures such as the phenolsulphonephthalein kidney function test, nonprotein nitrogen of the blood, blood urea, and blood sugar. As it stands the colorimeter cannot be used for other work such as blood creatinine, blood phosphorus, icterus index, bilirubin liver function test, and so on, without tedious calibration.

However, the instrument can readily be changed into a general colorimeter without additional expense. This can be accomplished simply by using an ordinary tuberculin syringe in place of the one which is provided with the instrument. It is then usable with the ordinary colorimetric formulas.

Two cubic centimeters of the standard solution is pipetted into the tube for the standard, and 1, 2, or 3 c.c. of the unknown color solution is pipetted into the unknown tube with the tuberculin syringe attached. Enough must be used so that the color is more intense than the standard. The solution is then drawn back into the tuberculin syringe until the colors of the standard and the unknown are matched in the eyepiece. The reading of the unknown will then be the amount originally pipetted into the unknown tube minus the reading on the tuberculin syringe, viz. 3.0 c.c. - 0.35 = 2.65 c.c. the reading of the unknown. The usual formula can then be followed as in the blood creatinine determination by the method of Folin.

$$\frac{\text{Reading of Standard}}{\text{Reading of Unknown}} \times 1.5 = \text{mg. creatinine per 100 c.c. blood which would be } \frac{2.00}{2.65} \\ \times 1.5 = \text{mg. creatinine per 100 c.c. blood}$$

The instrument with the tuberculin syringe used in this way has been checked against a large prism colorimeter and has been found to be accurate within the usual limits of error.

\*Received for publication, November 28, 1934.



TABLE III

THE INFLUENCE OF EXCESS OF SODIUM HYDROXIDE ON THE COLOR DEVELOPMENT

SAMPLE	CREATINE CONTENT	10% SODIUM HYDROXIDE ADDED	CREATININE DETERMINED	RECOVERY
		G.C.	MG.	%
1	Equivalent to 1 mg. creatinine	14.0	1.00	100
2	Equivalent to 1 mg. creatinine	14.2	1.00	100
3	Equivalent to 1 mg. creatinine	14.5	1.02	102
4	Equivalent to 1 mg. creatinine	15.0	1.00	100
5	Equivalent to 1 mg. creatinine	15.5	0.98	98
6	Equivalent to 1 mg. creatinine	16.0	0.98	98
7	Equivalent to 1 mg. creatinine	16.5	0.90	90
8	Equivalent to 1 mg. creatinine	17.0	0.90	90
9	Equivalent to 1 mg. creatinine	18.0	0.87	87
10	Equivalent to 1 mg. creatinine	20.0	0.78	78

TABLE IV

RECOVERY OF CREATINE ADDED TO URINE

SAMPLE	SOURCE	PREFORMED CREATININE	TOTAL CREATININE	CREATINE ADDED	CREATINE DETERMINED	
		MG./C.C.	MG./C.C.	MG.	MG./C.C.	%
1	Adult male	2.10	2.57	1.00	3.53	96.0
2	Adult male	1.95	1.93	1.00	2.91	98.0
3	Adult male	2.74	2.82	1.00	3.87	105.0
4	Child	0.78	1.00	1.00	2.00	100.0
5	Adult male	2.11	2.06	1.00	3.01	95.0
6	Adult female	2.72	3.45	1.00	4.46	101.0
7	Child	0.72	1.25	1.15	2.45	104.0
8	Adult male	1.06	1.43	1.15	2.61	102.6

Moderate variations in the temperature (from 0 to 25°) did not influence the values obtained.

In Table IV are given values which were obtained by analysis of several urines with the sulphuric acid procedure. Known amounts of creatine were added to the urines and attempts were made to determine the same. In each case the recovery was quantitative within the limit of accuracy of the colorimeter. The method has been used by us over a period of several months, and has been repeatedly tested as to its accuracy.

## CONCLUSIONS

A simple method for the analysis of creatine of urine by use of sulphuric acid is described. Extraneous red color, such as occurs in certain other procedures, is not formed, and creatine which has been added to urine may be accounted for quantitatively.

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## A MASS CULTURE APPARATUS FOR SECURING BACTERIAL CELLS FOR ANALYSIS\*

ROBERT A. GREENE, PH.D., TUCSON, ARIZ.

A RECENT study<sup>2</sup> of the chemical composition of the genus *Azotobaacter* has necessitated some means of securing large amounts of bacterial cells. Since the moisture content of these bacteria is approximately 85 to 90 per cent, a considerable quantity of the organisms must be "harvested" in order to secure sufficient material for any extensive chemical analysis.

A mass culture apparatus, as described by Vaughan<sup>4</sup> was not available, and neither were monel metal culture dishes of the type used by Huston, Huddleson and Hershey.<sup>3</sup> A very large number of culture dishes of the usual size were required to give any appreciable quantity of cells, so, after considering the various methods which have been compiled by Buchanan and Fulmer,<sup>1</sup> the following apparatus was used:

Pyrex glass trays, whose dimensions were 18 by 12 by 2½ inches were used as the culture dish. At first, enameled steel instrument trays were used as covers. Later copper covers, which were constructed locally, were used. These were more satisfactory, since they could be snugly fitted to the dish, and also because they were more resistant to hot air sterilization.

The cost of a dish and cover (in quantity lots) is approximately six dollars. Each dish had about 216 square inches of surface, which is readily accessible when the bacterial growth is to be harvested. Approximately 22 ordinary sized culture dishes (100 by 10 mm.) are required to give this area. At the present price of culture dishes (approximately 24 cents for routine grade, 36 cents for a good grade of resistance glass, and 50 cents for Pyrex glass), the cost of dishes to give the same surface as one large Pyrex dish would be slightly less expensive in the routine grade, but would be more expensive if dishes of resistance or Pyrex glass were used.

The mass culture dishes are sterilized in the hot air sterilizer in the usual manner; the media are prepared in large flasks, sterilized in the autoclave, allowed to cool to about 45° C., and then poured into the dishes. When the media has solidified, the dishes are inoculated by placing a small quantity of the bacterial suspension on the surface of the agar. This may be spread over the surface by tipping the plates, but preferably by smearing with a bent, flat-tipped glass rod, or a sterile swab.

At the end of the incubation period, the growth is scraped from the surface of the media, taking care not to remove any of the agar. A glass slide or a celluloid spatula<sup>5</sup> may be used. The material may be dried according to any

\*From the Department of Agricultural Chemistry, University of Arizona.  
Received for publication, December 1, 1934.

## A METHOD OF SPUTUM DIGESTION PRELIMINARY TO CULTURE FOR TUBERCLE BACILLI\*

W. L. McNAMARA, M.D., AND E. F. Ducey, M.D., WEST LOS ANGELES, CALIF.

DIGESTION is a vital step preliminary to the culture of tubercle bacilli from biologic specimens such as sputum, urine, feces, and exudates. This must accomplish the killing of extraneous organisms, as well as the freeing of the tubercle bacilli from caseous masses. While digestion may be done in several different ways the use of either acids or alkalies is the method most generally employed at present. Pinner recommends equal parts of sputum and 3 per cent hydrochloric acid for a period of thirty minutes. Petroff uses equal parts of sputum and 3 per cent sodium hydroxide from thirty to forty minutes. We have found that when the period of digestion with acids or alkalies is extended for four to six hours many of the tubercle bacilli are killed. This suggests the possibility that a certain number of organisms may be killed within a minimal period of time, a factor of importance in specimens in which the total number is small.

We employ the following method for digestion, and have found that it is entirely safe, having recovered viable organisms often after seventy-two hours of digestion in the incubator; the sputum is completely liquefied and freed from small caseous masses which may harbor spores.

*Method of Digestion:* 10 c.c. of sputum is placed in a 50 c.c. centrifuge tube (sterile), 20 c.c. of 1 per cent hydrochloric acid and 100 mg. of pepsin (Stearns 1-12,000) are added. The tube is closed with a rubber stopper which is secured with rubber bands or adhesive tape to prevent leakage. The tube is placed in a sputum shaker or Kahn shaker for fifteen minutes and is then placed in the incubator for twelve hours. It is then neutralized to litmus with normal sodium hydroxide and centrifugated for one hour.

Tubes and plates are inoculated with material aspirated from the bottom of the tube with a sterile capillary pipette.

With organisms recovered by this method of digestion we have been able to reproduce tuberculous lesions in guinea pigs which were verified by histologic examination, and have obtained typical growths on culture media. We feel that the greater amount of time consumed by this method is more than compensated by the fact that the danger of killing the tubercle bacilli is reduced to a minimum.

\*From the Clinical Laboratory, U. S. Veterans Administration Facility.

Received for publication, November 30, 1934.

Published under R. & P. 6969, U. S. Veterans Administration.

## A SPECIAL CAMERA FOR ROUTINE PHOTOMICROGRAPHY\*

J. A. REYNIERS, M.S., NOTRE DAME, IND.

FROM time to time, pocket or box cameras have been suggested for use in photomicrography. Most of these are makeshift devices intended for emergency use or as a substitute for expensive apparatus.

There is in photomicrography, as distinct a need for a small camera which can be manipulated quickly and which will allow inexpensive exposures to be

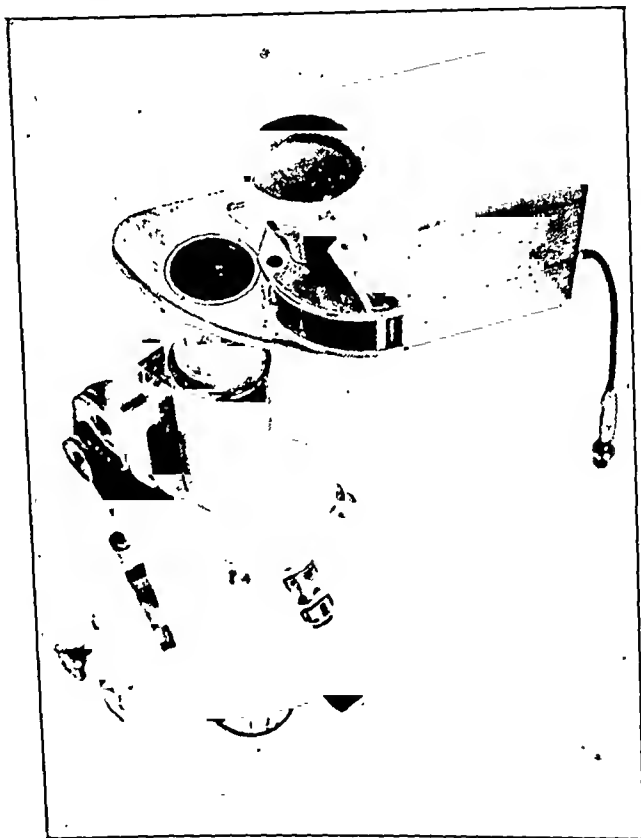


Fig. 1.

made, as there is need for a plate camera. Obviously, a film camera with a small-sized picture frame is not as satisfactory in some instances as a plate camera but on the other hand the inconvenience and expense of using a plate camera in routine work are factors which must be considered. It goes without comment that exact pictures can be obtained with small-sized-film negatives which can then be enlarged.

\*From the Laboratory of Bacteriology, University of Notre Dame.  
Received for publication, December 1, 1934.

standard method, but I have found that desiccation in anhydrous acetone, as suggested by Wilkerson and Gortner<sup>5</sup> is very satisfactory.

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### A VASELINE DISPENSER FOR HANGING DROP PREPARATIONS\*

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ROBERT A. GREENE, PH.D., TUCSON, ARIZ.

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NUMEROUS methods have been used to dispense vaseline in the preparation of hanging drop mounts, but many of these are not especially satisfactory, particularly for use in crowded student laboratories. The following method has been used in the Soil Microbiology Laboratory at the University of Arizona: an empty tube, which has a long, slender "nozzle" is packed with vaseline, and is used to dispense the vaseline for the hanging drop preparations. The types of containers used for nasal ointments are satisfactory, and may be secured from druggist's supply companies. The use of such a dispenser in a student laboratory has resulted in neater hanging drop preparations. \*

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\*From the Department of Agricultural Chemistry, University of Arizona.  
Received for publication, December 1, 1934.

# AN ELECTRODIALYSIS APPARATUS FOR USE WITH ALTERNATING CURRENT\*

E. J. CZARNETZKY, PH.D., SAN FRANCISCO, CALIF.

ALTHOUGH there are several types of apparatus described in the literature, they are as a rule expensive and not entirely satisfactory. The present set-up is inexpensive and the parts are readily replaceable. It has the additional advantage of ease in changing the membranes without disturbing the solution to be dialyzed.

The electrodes are carbon or graphite rods which are placed close together to facilitate rapid ionic transfer, leaving room for a large volume of solution. Where metal salts, such as silver, are to be dialyzed, gold electrodes can be used on the negative side so that the metal is plated directly on the electrode, and thus furnish a measure of the extent of dialysis by determining the difference in the weight of the electrodes before and after plating. Various grades of DuPont cellophane can be used for the membranes. The cellophane is attached to the electrode cylinder by means of DeKhotinsky cement.

Fig. 1 shows the details of the apparatus. The ammeter is connected in shunt and can be connected by throwing the switches. If two 250 watt lamps are used in the eleats *I* and *J*, there will be approximately five amperes traveling through the system, provided it is not held back by the solution being dialyzed. The lamps also serve as indicators for the continuity of the system. Usually about 50 volts are required, and five amperes to start. As the concentration of electrolytes in the solution *Q* becomes less and less, the amperage will fall. When it has reached a figure close to zero, the dialysis is complete. The amperage is controlled somewhat by the rectifier. The latter consists of an electrode of tantalum metal of large surface and one of lead in a solution of sulphuric acid. The sulphuric acid coats the tantalum with a unimolecular layer of oxide, which is unipolar, and therefore only carries current in one direction. (Tantalum can be bought for about \$1.50 per gram from the Fansteel Company, North Chicago, Ill.) It is necessary to use insulators as indicated, so that the current can be forced through the solution *Q*.

When the substance to be drawn out of the mixture is a simple salt such as sodium chloride, the amount of salt can be determined by titrating the solution in container *N*. This gives equivalents of base, and thus equivalents of monovalent salts. Cooling is provided by the flow of dialyzing water through *O* and *P*.

The flow of water from containers *F* and *G* should be slow so as to leave enough electrolyte in the system to carry the current. The dialyzing chambers

\*From the Research Laboratories of the Mount Zion Hospital.  
Received for publication, December 7, 1934.

The camera described in this paper has been designed for a definite purpose, i.e., routine photography. It has been in use for over two years in this capacity and has been most satisfactory. It is attached directly to the microscope in such a manner that a single motion will swing it aside so that there is no interference with the normal use of the microscope. The operator can see the exact field that he wishes to photograph and can focus the camera with precision. The entire apparatus is mechanically exact and very light.

The apparatus, Fig. 1, consists of a small film box built to use regular pocket size film and capable of taking 16 exposures on a standard 8 frame roll. The film is held perfectly flat against a spring. The box is mounted on an arm which is made from thin metal and pivoted to another arm which can be attached directly to the tube of the microscope by a special self-centering collar. This arrangement permits the upper arm bearing the camera to be swung from side to side. A viewing device is mounted next to the camera. A strip of film is used to screen the image. The diameter of this viewing device is such that it will cover an entire field as projected by a 10X ocular. Film is used in place of the usual ground glass because of its extremely fine-grained surface. The whole outfit is rigidly constructed. To be sure that the viewing device and the film are on exactly the same plane, the upper arm is guided by a groove in the lower arm. Because of the lightness of the camera and its particular construction, it does not bear directly on the tube of the microscope, consequently it does not interfere with the focus.

The camera remains attached to the microscope and is swung to one side when not in use. When a field is photographically desirable the image finder is brought into place and is checked by a click stop; the image is focused sharply and the light adjusted. The camera is moved into position and is held by a similar stop. The image is then photographed after which the camera is again pushed out of the way and observations are resumed. So easy is this motion that an operator familiar with photographic conditions and using a fast film can scan and photograph living forms with reasonable success.

This device has a wide range of usefulness and is especially valuable in the hospital or pathologic laboratory as well as in routine bacteriology or cryptogamic botany.

# A NOTE ON THE DETERMINATION OF UREA IN BLOOD BY THE FOLIN DISTILLATION METHOD\*

## DETERMINATION OF UREA BY STEAM DISTILLATION

F. H. L. TAYLOR, AND MARGARET A. ADAMS, BOSTON, MASS.

THE steam distillation method described below was developed for the single purpose of eliminating the close supervision required by the present Folin-Wu<sup>1</sup> urea method and the modification of Lewis<sup>2</sup> or the newer method of Peskett.<sup>4</sup> To the many workers in the biochemical field, using the distillation method for urea, it is presented as a modification which has been used with success in many determinations of urea during the past two years and which eliminates the technical difficulties of backsucking of the distillate and too rapid evolution of ammonia. These two difficulties are often extremely difficult to eliminate in the older Folin method even with the closest attention. The modification includes the precaution of cooling the distillate (presented by Lewis) which has been used routinely in this laboratory as a useful precaution for some years.†

*Apparatus.*—A diagram of the apparatus is shown in Fig. 1. A = Erlenmeyer flask containing distilled water which has been boiled for about ten minutes. A two-hole rubber stopper in flask A contains: B, a short straight piece of glass tubing at the top of which is a 2-inch piece of rubber tubing and a pinch clamp; and C, which is a glass delivery tube of about 7 mm. bore leading from the flask A to the bottom of the distilling tube D. C may be one piece of glass tubing with angles in two places, or it may be composed of two glass bends connected by a short piece of rubber tubing, provided the ends of the two pieces of glass tubing are in contact with each other.

D is a Pyrex ignition tube containing the material in which the urea N is to be determined. By means of a two-hole rubber stopper in the neck of D, a delivery tube leads into the receiving tube F.

E is best prepared from a 5 or 10 c.c. volumetric pipette, since the bulb of the pipette still further decreases the possibility of sucking back from F into D. The pointed end of the pipette may be removed to make the delivery tube the desired length, but it is advisable to fire-polish the delivery end to slightly decrease the bore of the tube. A slight bend is made in the bottom of E so that the end of E will always be beneath the surface of the liquid in the receiving tube F.

\*From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital and the Department of Medicine, Harvard Medical School. Received for publication, December 13, 1934.

The expenses of this research were paid in part by a gift to Harvard University from Smith, Kline and French Laboratories of Philadelphia.

†The reagents and general procedure used are taken directly from Folin's outline of procedure.<sup>3</sup>



*O* and *P* should have their side arms near the top of container *R* so that extra water is not forced into the solution *Q* by hydrostatic pressure. Where the amount of solution is small, use a smaller vessel, *R*.

Where ampholytes are used there will be some interference by reason of the Donnan effect, but this is overcome finally by the force of the current, so

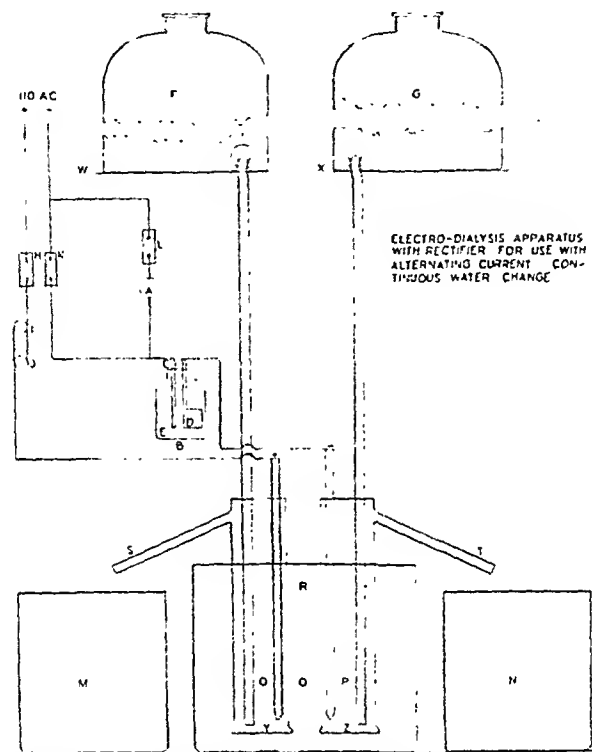


Fig. 1.—*A*, Ammeter in shunt. *B*, Tantalum rectifier. *C*, Lead electrode. *D*, Tantalum electrode with large surface. *E*, Five per cent sulphuric acid electrode solution which oxidizes the tantalum electrode with a unimolecular coat of oxide, which is unipolar and does not emit electrons. *F*, and *G*, Large glass jars containing distilled water which act as reservoirs with outlets leading to the electrode cylinders. *H*, Switch to start and stop current. *I* and *J*, Two pony fuses for holding lamps for the purpose of furnishing resistance to the current. They are lit if it is a sign that current is flowing through the solution being dialyzed. *K*, and *L*, Switches for connecting the ammeter (*A*) in the line. *M* and *N*, Reservoirs for catching the spill over waste solutions which come from the dialyzing chambers. *M* contains waste acid as it is the positive pole. Conversely, *N* contains alkali as it is the negative pole. *O* and *P*, Electrode cylinders made of Pyrex glass. *Q*, The solution to be dialyzed. *R*, Glass jar for holding the solution to be dialyzed. *S* and *T*, Side arms for conducting the waste fluids to the reservoirs, *U*, Glass or rubber plate for insulation. *W* and *X*, Glass or rubber plates for insulation. *Y* and *Z*, Cellophane membranes covering the open ends of the dialyzing cylinders.

that little if any electrolyte remains in the solution. When dialyzing haptenes and substances of that nature attached to proteins, use a smaller current so that the compounds are not pulled apart.

**Caution:** When making the rectifier, leave an air hole in the top so that  $H_2$  and  $O_2$  formed by electrolysis can escape.

Prior to commencing a series of determinations the water in the flask *A* should be boiled for ten minutes. A portion of the steam emitted should be tested for ammonia by direct Nesslerization. This precaution renders unnecessary the use of nitrogen free water which was originally used in this method.

Water in flask *A* must be kept boiling at an even rate throughout a determination. The use of a few glass beads in the bottom of the flask is desirable to prevent bumping.

In using a microburner to heat the tube *D*, care must be exercised not to heat too vigorously. The rate of admission of steam from *A* must be sufficiently rapid to prevent sucking back of the distillate in *F*. Once an even, moderately fast boiling has been established in the flask and the distilling tube, there is no necessity of watching the distillation continuously.

The apparatus is washed by steam before use by placing an ignition tube containing a little boiling water in place of *D*.

TABLE I

A COMPARISON OF THE STEAM DISTILLATION METHOD WITH THE ORIGINAL FOLIN-WU METHOD  
(Values in mg. of Urea Nitrogen per 100 ml. Blood)

NO.	FOLIN-WU	STEAM DISTILLATION
1	10.78	10.80
2	13.60	13.80
3	15.38	15.34
4	18.90	18.80
5	(15.9 )*	(15.80)*
	(16.1 )	(16.00)
6	(13.3 )*	(13.80)*
	(13.3 )	(14.10)
7	(14.20)*	(14.40)*
	(14.50)	(14.30)
8	(11.50)*	(11.90)*
	(11.70)	(12.10)

\*Duplicate determinations.

## RESULTS

Table I presents data comparing the results obtained by this modification with those of the original Folin-Wu method.

## SUMMARY

1. The Folin-Wu distillation procedure for blood urea is modified by the inclusion of a steam distillation apparatus.
2. The values obtained by the method are in close agreement with those obtained by the original Folin-Wu procedure.
3. The modification has the advantages of freedom from close technical supervision and eliminates most of the hazards of the original procedure.

## REFERENCES

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2. Lewis, Robert C.: A Note on the Distillation of Ammonia in the Folin-Wu Method for the Determination of Urea in Blood, *Am. J. Clin. Path.* 1: 227, 1931.
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4. Peskett, G. L.: *Brit. J. Exper. Path.* 15: October, 1934.

*F* is a test tube graduated at 15 and 25 c.c. During the distillation, *F* should be immersed in a beaker *G* containing ice water or water kept cool by constant flow.

*Procedure.*—Transfer 5 c.c. of tungstic acid blood filtrate to a Pyrex ignition tube (*D*). This tube must be rinsed with  $\text{HNO}_3$  and water if it has previously contained Nessler's solution. Add 2 drops of buffer mixture and 1 c.c. of urease solution or a piece of urease paper. Stopper, and immerse the tube in warm water ( $37.5^\circ \text{C.}$ ) for fifteen minutes. Transfer 2 c.c. of 0.05 N-HCl to a test tube graduated at 15 and 25 c.c. (receiving tube *F*). Attach the receiving tube to the distilling apparatus in such a way that the end of the delivery tube *E* is under the surface of the liquid in receiving tube *F*, add to tube *D* a glass bead, 2 or 3 drops of paraffin oil, and 2 c.c. of saturated borax solution, and attach the tube so that the end of *C* is under the surface of the liquid in *D*. The tube *D*

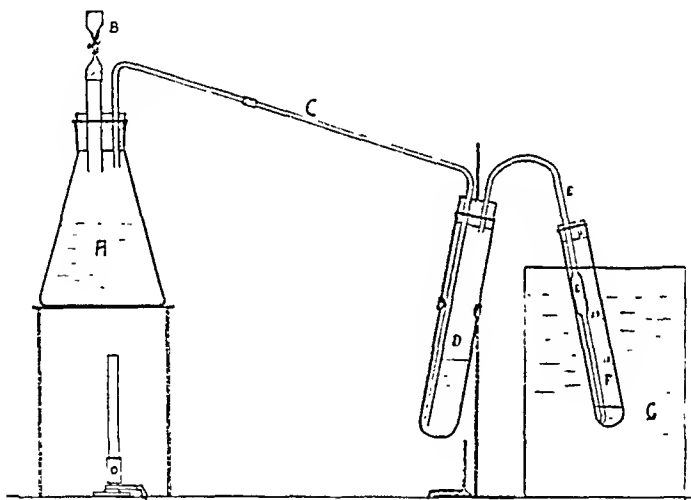


Fig. 1.

should not be inserted in the apparatus until all other parts of the apparatus are in readiness for immediate distillation. The water in *A* should have been brought to boiling previously, with the pinch clamp *B* open. By adjusting the pinch clamp on *B*, send the desired amount of steam over into *D* until the liquid in *D* is caused to bubble. A microburner may be used to hasten boiling in *D*, and may then be placed in such a way as to warm the sides of tube *D*, thus lessening condensation of steam. When the liquid in *F* has risen to the 15 c.c. mark, slip off the receiving tube *F* from the rubber stopper, and let it rest in a slanting position while the distillation is continued for one minute more. Rinse the lower end of the delivery tube with a little water, cool the distillate and dilute to about 20 c.c. Transfer 0.3 mg. N to a 100 c.c. volumetric flask, dilute to about 75 c.c. Add 10 c.c. Nessler's to standard and 2.5 c.c. to unknown. Make to volume and read in colorimeter with standard set at 20 mm.

*Precautions.*—All rubber stoppers and connections must be boiled to remove  $\text{NH}_3$  before using.

following day, from 6 to 10 c.c. of blood are withdrawn by means of a vacuum tube from the heart of one of these rabbits, the blood is allowed to clot, the serum is removed, tested for sterility, and the patient is then inoculated subcutaneously or intramuscularly with this serum. Inoculations with the vaccine of the causative organism are continued daily with the series of rabbits already immunized and which have been selected for use in the particular case under treatment. If necessary the serum from the blood of these rabbits in rotation is administered to the patient on successive days.

The patient's blood is directly matched for a compatible donor. It is of advantage to have this procedure completed on the earliest indication that the case may prove to be one of septicemia. The suitable donor reports to the laboratory and from 60 to 100 c.c. of blood are withdrawn into a large vacuum tube. The blood is allowed to clot at room temperature, and subsequently placed in the ice chest for twelve to eighteen hours. The serum is removed and the patient is transfused with this serum. If considered necessary, this procedure is also carried out daily, in which case it may be advisable to have more than one donor. As a rule Cadham has administered the human serum on alternate days. The average number of combined treatments administered to the patients was 4, the greatest number administered to any single patient, 10.

No drugs were given during the course of the treatment. On occasion, whisky was prescribed as a stimulant and then sparingly. The patient was encouraged to partake of a fairly liberal diet.

Serum treatment was instituted on the average on the sixth day following the initial symptoms; the earlier administration was on the third day, the most delayed on the sixteenth day. The first treatment with serum administered to a patient with subacute bacterial endocarditis was six weeks subsequent to the initial symptoms.

**PREGNANCY, Conservative Treatment of Late Toxemias of, Mc Nelle, L. G. J. A. M. A. 103: 548, 1934.**

In 799 cases of late toxemias of pregnancy, 259 were of the eclamptic type.

There was a definite decrease in the mortality in eclamptic patients treated by conservative methods at the Los Angeles County General Hospital.

The intravenous injection of a 10 per cent solution of magnesium sulphate has proved a very valuable adjunct in the treatment of the late toxemias of pregnancy. It will cause some reduction of blood pressure, reduce edema, increase urinary output and reduce or control other symptoms.

The intravenous injection of a 10 per cent solution of magnesium sulphate will control the convulsions of eclampsia in nearly every case, and exercises a favorable influence on the other symptoms of eclampsia.

The intravenous injection of a 10 per cent solution of magnesium sulphate is clinically proved to be a safe procedure.

**ANEMIA, Classification and Treatment of, on the Basis of Differences in the Average Volume and Hemoglobin Content of the Red Corpuseles, Wintrobe, M. M. Arch. Int. Med. 54: 256, 1934.**

Observations on the volume and hemoglobin content of the erythrocytes of more than 1,000 healthy and anemic persons are recorded and discussed under the heads of accuracy of the methods employed, normal values, physiologic variations and variations in disease.

The anemias may, on the basis of differences in the mean volume and hemoglobin content of the red corpuseles, be subdivided into four groups. Each of these groups appears to correspond to a fundamentally different type of disturbance in the hematopoietic apparatus. (1) Macrocytic, (2) Normocytic, (3) Simple microcytic, (4) Hypochromic microcytic.

Consequently the method of classification proposed is useful as an aid to diagnosis. It facilitates also the separation of conditions in which liver therapy may be expected

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**POISONING, Gasoline and Kerosene, in Children, Nunn, J. A., and Martin, F. M. J. A. M. A. 103: 472, 1934.**

There appears to be no specific antidote for gasoline or kerosene poisoning. Treatment consists of removing as much of the offending agent as possible by gastric lavage or emesis and laxatives. During gastric lavage there is much retching, struggling and breath catching, which seem to favor aspiration of stomach contents into the lungs. It is the authors' impression that there is less likelihood of aspiration of the fluid when emesis is produced by the oral administration of syrup of ipecac than when gastric lavage is employed. In their more recent cases when cyanosis and other signs of respiratory embarrassment are present, they believe that the use of oxygen (95 per cent) and carbon dioxide (5 per cent) has been of considerable benefit. They therefore recommend the administration of oxygen and carbon dioxide in all cases that show such signs. It is employed for its stimulating action on the respiratory center rather than to increase the oxygen-carrying power of the blood. They have also given atropine sulphate and caffeine sodiobenzoate hypodermically for their stimulating effects.

**DYSENTERY, Sonne, Felsen J., and Osofsky, A. G. J. A. M. A. 103: 966, 1934.**

Sonne dysentery chiefly affects children and is characterized by a short incubation period, brief course and general mildness.

The disease exists in endemic and epidemic form in the United States.

With increasing incidence it is expected that the general character of the disease will change and, in epidemic form, affect more adults and cause a higher degree of mortality in children.

Sonne dysentery is a contact infection and outbreaks can be terminated by proper epidemiologic measures.

The effects of high titer serums in patients and of specific vaccines in contact cases are now being studied.

**SEPTICEMIA: A Method of Treatment, Cadham, F. Am. J. M. Sc. 188: 542, 1934.**

A method is described for the treatment of septicemia by inoculation of a specific antiserum and by transfusion of normal human blood serum. A brief report of 100 cases is submitted: 85 of the patients recovered. Excluding the 5 cases of subacute bacterial endocarditis, all fatal, the mortality rate of the other 95 cases was 10.5 per cent.

Numerous strains of streptococci and staphylococci are recovered from patients with septicemia or from patients with pyemic foci resulting from infection with these bacteria. The microorganisms are cultured in serum broth and a vaccine prepared. A number of rabbits are selected and inoculated with a heavy suspension of the vaccine. Vaccine is administered to the rabbit when six months old and the inoculations continued twice weekly throughout the useful life of the rabbit, using numerous strains of streptococci and staphylococci. The subcutaneous and intravenous route are both employed. When one of these animals is two years of age it is considered suitable for the immediate purpose in view. Cadham keeps 25 rabbits continuously immunized.

When a positive culture is obtained from a patient showing symptoms of septicemia, a vaccine of the microorganism is prepared as rapidly as possible (generally within twenty-four hours), and this vaccine is inoculated into a series of the immunized rabbits. On the

In which  $R_r$  is the "area of reticulocyte response" in square centimeters and  $E_0$  the initial erythrocyte count (millions per c.mm.).

By the use of these formulas curves were prepared for the "area of the reticulocyte response" similar to those devised by Riddle and by Bethell and Goldhamer for the maximum reticulocyte percentage response, in terms of the initial red blood cell level.

**HYPOGLYCEMIA, In Diabetes Associated With Obstruction of Pancreatic Duct, Zeckwer, I. T. Arch. Int. Med. 188: 330, 1934.**

A patient with a history of diabetes but no medication with insulin manifested hypoglycemia. At autopsy, calculi obstructed the pancreatic duct, with resultant extraordinary atrophy of the acini and islands; the liver showed degenerative changes, and there was pulmonary tuberculosis.

The clinical condition paralleled experimental pancreatectomy in the dog, in which it has been shown that hepatic insufficiency results from deprivation of the external secretions of the pancreas.

Points of similarity between the clinical case and the experimental animal were: (1) the development of hypoglycemia after the hyperglycemic period and (2) hepatic degeneration. The latter, however, was of a minor degree and of a different gross and histologic character from that seen in dogs.

The factor of undernutrition must be considered in the production of the hypoglycemia in this case, but has less experimental basis than the effect of loss of external pancreatic secretions.

In 10,300 autopsies performed in this department, only 4 other instances of pancreatic calculi could be found, none of which was associated with diabetes.

It is suggested that when the liver is damaged through loss of the external secretions of the pancreas, especially if the condition is aggravated by undernutrition and tuberculosis, hypoglycemia may result in diabetes even in the absence of treatment with insulin.

**INFLUENZA, Epidemic, A Note on the Suggested Epidemiological Relationship of Certain Strains of Green Streptococci to, Noble, W. C., and Brainard, D. H. Am. J. Hyg. 20: 191, 1934.**

Several workers have reported the isolation of specific strains of green streptococci from the throats and blood of influenza patients. Falk claims that he has repeatedly isolated strains identical with his 42xS and 18dR from human influenza material, and from monkeys inoculated with this material. The authors have studied the agglutination of a large number of strains which they have isolated from influenza patients and from other sources. None of these strains is agglutinated by the antiserum for 18dR. Some of the strains cross-agglutinate in unabsorbed serum with 42xS; they may therefore bear group relationship to 42xS, but they are certainly not identical with it. It should be noted that they were isolated as frequently from noninfluenzal as from influenzal sources, three of them being isolated from normal, uninoculated monkeys.

Rosenow and Falk state that they have found agglutinins for their strains in the serum of influenza patients. The serum from none of our influenza patients or convalescents contained agglutinins for either 42xS or 18dR.

The authors have therefore found no evidence that either of these organisms is the cause of influenza.

**AGRANULOCYTIC ANGINA, Following Ingestion of Dinitrophenol, Bohn, S. S. J. A. M. A. 103: 249, 1934.**

Agranulocytic angina developed in a patient after the ingestion of 21.8 gm. of 2, 4 dinitrophenol sodium over a period of four months.

The dosage was 4 mg. per kilogram of body weight daily for two weeks and then doubled until the onset of unfavorable reactions.

Treatment consisted of discontinuing the drug, administration of pentnucleotide and one transfusion of 250 c.c. of whole blood, following which the patient recovered.

to be of value from those in which treatment with iron preparations is successful, and distinguishes these two types of anemia from those in which neither method of treatment can be expected to succeed.

**JAUNDICE, Hemolytic, Mechanism of Increased Fragility of Erythrocytes in, Haden, R. L. Am. J. M. Sc. 188: 441, 1934.**

The erythrocytes in congenital hemolytic jaundice constantly exhibit spherocytosis, best shown by the increased volume-thickness index.

The diameter of the red cell in this condition is always less than normal; the volume is variable.

When placed in hypotonic salt solution, normal erythrocytes become progressively more globular with little change in diameter as the solution is made more hypotonic.

There is a direct relation between the volume-thickness index and the fragility of red cells.

In congenital hemolytic jaundice, the erythrocytes have at the beginning one of the shapes through which a normal cell must pass when placed in successive dilutions of hypotonic salt solution, and so may be regarded as nearer the hemolysis point.

The one fundamental variation from normal in congenital hemolytic icterus is the microspherocytosis. The anemia, jaundice, splenomegaly, reticulocytosis and increased fragility are all secondary to the globular form of the erythrocyte.

**AUTOHEMAGGLUTINATION, Sherman, I. Am. J. M. Sc. 188: 487, 1934.**

Three cases are reported in which autoagglutination of the red blood cells occurred at room temperature and disappeared at body temperature.

The phenomenon is due to the presence of agglutinins in the serum which produce clumping of red blood cells of all four blood groups at temperature below a critical point. This point is between 30° and 31.5° C.

Two of the cases reported belong to Group 0 and 1 to Group A.

All 3 cases were suffering with a febrile infectious condition. Reexamination in one case, after recovery of the patient, showed diminution of the agglutinating power of the serum to an extent that the clumping of the red cells occurred only in the ice box but not any longer at room temperature.

Two of the 3 patients received blood transfusions 5 and 2 times respectively, without any untoward reaction.

**ANEMIA, Pernicious, Relation Between Total Reticulocyte Count and the Degree of Bone Marrow Involvement, Bethell, F. H. Am. J. M. Sc. 188: 476, 1934.**

In an effort to estimate the comparative quantities of reticulocytes produced during the initiation of a remission in different pernicious anemia patients, the areas circumscribed by the graphs of the "reticulocyte responses" plotted on a standard paper, were measured in square centimeters.

The value so obtained for any patient is an expression of the total reticulocyte response of that patient.

From the data obtained, formulas were constructed by which a measure of the expected total reticulocyte response can be secured from a knowledge of the initial red blood cell count.

$$\text{For liver extract by mouth} \quad R_s = \frac{67.37}{0.369} \text{ sq. cm.} \\ E_o$$

$$\text{For desiccated stomach} \quad R_r = \frac{86.93}{0.385} \text{ sq. cm.} \\ E_o$$

This is in complete agreement with the previous studies. Since it is a specific test, unlike the nonspecific white blood cell count or fever and pulse chart, it would seem to merit as much as these methods in the diagnosis of active tuberculosis. It is very unlikely that such weak dilutions will produce other than skin reactions. Ayman has not encountered a single focal or general reaction after the 1:50,000 dilution.

With the recent availability of the crystalline tuberculo-protein, it should soon be simple to determine once for all a dilution of this tuberculo-protein which will give these reactions. Then such a dilution will always be the one to use, whereas at present the proper dilution of the old tuberculin will have to be determined for each batch.

**BACTERIAL CARBOHYDRATES, Serological Specificity, With Special Reference to Type II Pneumococcus and a Heterophile Strain of Bacterium Lepisepticum, Dingle, J. H. Am. J. Hyg. 20: 148, 1934.**

Specific carbohydrate substances have been isolated from Types I, III and III *Diplo. pneumoniae*, *Past. cuniculicida*, *Sacch. cerevisiae*, *E. coli*, and *P. marginalis*, and the method of preparation has been outlined.

The carbohydrate substances so isolated have been found to react specifically in high dilution with homologous immune sera and to be free from protein as evidenced by the biuret reaction.

The carbohydrate substances of *Past. cuniculicida* and *P. marginalis* isolated from these organisms for the first time, were found to be soluble in aqueous or saline solution, to react with homologous antisera in high dilution, to give positive Molisch reactions and negative biuret tests, and to be nitrogen-free. They did not reduce Benedict's solution nor did they give a color with iodine.

Determinations of the specificity of the bacterial carbohydrates were made by carrying out precipitin reactions with solutions of the carbohydrate substances and antisera against a variety of bacteria.

The cross-reacting precipitations occurring with the polysaccharides from Types I, III, and III pneumococcus and antisera against various bacteria were for the most part slight.

A pronounced characteristic carbohydrate precipitate, however, was obtained with rabbit antisera for the heterophile *Past. cuniculicida* 370 with Type II pneumococcus carbohydrate. Slight cross-reactions were also noted between the *Past. cuniculicida* carbohydrate and both Type II antipneumococcus rabbit serum and Type III antipneumococcus horse serum.

The polysaccharide from *Sacch. cerevisiae* did not give cross-reactions with Type II antipneumococcus serum as had been reported by Sugg and Neill, but did give slight reactions with antisera against *P. marginalis*, Friedlander's bacillus, and *Past. cuniculicida* 370.

Reactions of almost uniform intensity were noted between the carbohydrate from *Sacch. cerevisiae* and rabbit immune sera against the seeds of lettuce, squash, beet, wheat, cabbage, sunflower, alfalfa, carrots, corn, peas, and beans.

Some variation in the intensity of the reactions of *Past. cuniculicida* 370 carbohydrate with homologous sera from different rabbits was noted, but no reaction at all was given with the antiserum for the avirulent strain, RD3.

The carbohydrate isolated from a fecal, encapsulated strain of *E. coli* reacted specifically with its homologous antiserum but with no others. It did not react with an antiserum for a strain of nonfecal *E. coli*.

The carbohydrate from *P. marginalis* showed slight reactions with Types II and III antipneumococcus horse sera and corn and wheat antisera. In determining the specificity of this substance, it was found that three of the seven antisera for organisms of the genus *Phytomonas* showed slight reactions: *P. marginata*, and the hop and daisy strains of *P. tumefaciens*.



**TUBERCULIN, Skin Sensitiveness to, in Primary Tuberculosis, Stewart, C. A. J. A. M. A. 103: 175, 1934.**

The average degree of skin sensitiveness to old tuberculin present in a group of infected children with negative or inconclusive chest films was essentially equal to that present in groups of cases with definite and conspicuous demonstrable intrathoracic lesions in different stages of resolution and considered characteristic and typical of tuberculosis of first infection. The mean level of allergy to tuberculinoprotein produced in groups of children by chance infections is independent, therefore, of the absence, presence, scarcity, abundance, size or character of the primary tuberculous lesions demonstrable by roentgen examinations during life.

The distribution of different degrees of skin sensitiveness to old tuberculin in a group of infected children consisting of cases with normal or inconclusive chest films was found not to differ essentially from that present in other cases with distinct demonstrable intrathoracic lesions deemed characteristic of tuberculosis of first infection. The curves that represent the general pattern of the distribution of different degrees of allergy to tuberculinoprotein found in each group of cases are very similar.

This study on living patients failed, therefore, to disclose evidence that justifies one in entertaining suspicions that children with large and intense Mantoux reactions harbor more extensive pathologic changes or have more active primary tuberculous lesions than do other children who are less sensitive to tuberculin.

Groups of children with primary tuberculosis exclusively, who are dissimilar roentgenologically, are alike as far as the average group skin sensitiveness to tuberculin is concerned. Apparently soon after tubercle bacilli make their initial entry into the body and before the primary tuberculous lesions have time to resolve, allergy reaches an average group level that is sustained with little or no definite change throughout the first few years that postdate the primary infection.

Changes in the size of Mantoux tests throughout the periods of increment and decline of the reactions in children with normal or inconclusive chest films roughly parallel similar changes manifested by skin reactions in other children with definite demonstrable intrathoracic lesions typical of tuberculosis of first infection.

**TUBERCULIN TEST, The Intracutaneous Quantitative in the Diagnosis of Active Tuberculosis, Ayman, D. J. A. M. A. 103: 154, 1934.**

It seems clear that with a given batch of old tuberculin it is easily possible to determine a dilution of this which will give a minimum of positive reactions of a certain size in patients without active tuberculosis and a maximum of such positive reactions in patients with active tuberculosis. More specifically, in the present study a 1:50,000 dilution of old tuberculin injected into 202 nontuberculous patients was found to give 97 per cent negative results; i.e., reactions less than 10 by 9 mm. in size. The same dilution injected into 82 patients with active tuberculosis gave 69 per cent of positive reactions 10 by 9 mm. in size, and when severely ill patients with high fever and bronchopneumonic areas in the lungs were subtracted from the total tuberculous group, 93 per cent of the tuberculous patients gave adequate positive reactions 10 by 9 mm. or more in size. In seriously ill patients it is known that the allergic responses are diminished or absent, and since all these patients had every symptom and sign of active tuberculosis, their diagnosis is obvious without the dilute tuberculin test. It therefore seems valid to subtract these seriously ill patients from the total group. Also, the fact that such dilute reactions occur chiefly when the patient is only mildly or moderately ill rather than moribund would appear to enhance the value of the test, for the difficulty in the diagnosis of tuberculosis is greatest in those patients who are not yet seriously ill.

With only 3 per cent of clinically nontuberculous patients reacting to the 1:50,000 dilution with areas 10 by 9 mm. or more, and from 69 to 93 per cent of such positive reactions in patients with active tuberculosis (depending on whether one includes seriously ill and moribund patients), Ayman believes that such a test has definite diagnostic value.

The section on the preparation and examination of smears from the mouth—referred to in the book as “Microscopic Examination of the Oral Cavity,” is, it seems to this reviewer, somewhat sketchy. The excellent work of Keilty in this field in which these methods are well worked out, is not referred to; the Weiss stain for Vincent’s organisms might well be included; the method of staining tubercle bacilli in the cold has long supplanted the use of heat; and, while *B. leprae* is acid-fast, decolorization must be carried out with more care than when staining for tubercle bacilli.

Incidentally, the book is not restricted to a consideration of diseases of the mouth per se but includes also a discussion of the changes seen in the mouth in disease in general thus rounding off the subject.

This book can be recommended as a useful contribution. The numerous illustrations are excellent and helpful.

### Handbook of Anesthetics\*

THE fourth edition of this well-known and excellent little book has been thoroughly revised to include the new methods and new apparatus which have established a definite claim to recognition.

As before, this book remains an authoritative and comprehensive presentation of anesthesia and may be regarded as a safe guide to technique.

### Recent Advances in Endocrinology†

ENDOCRINOLOGY has come to be accepted as a term indicating the science concerned with the study of internal secretion, by which is meant the products of those glands which separate within themselves specific compounds and secrete them generally into veins, very occasionally into lymph vessels.

Endocrinology is a relatively new development and as such has suffered from a confusion of ideas and a still more marked confusion arising from a more or less haphazard terminology. As Cushing has aptly said: “Endocrinology lends itself to two glaring faults, one the popularization of writing on the subject, and the other a tendency of clinical observers to draw upon their fancy in a symptomatology which does not lend itself to precision.”

Under these circumstances it is not to be wondered at that the clinician, even though he be interested in the subject, should have difficulty in keeping pace with the increasing studies made and reported in this field, and still more difficulty in assigning to each its proper value and importance.

For this reason, the first edition of Professor Cameron’s book, presenting as it did a sane, comprehensive, and careful review of this difficult subject, was a welcome addition to the medical library.

Equally as welcome is this second edition which takes cognizance of and includes the studies in endocrinology up to August, 1934. As a consequence this book has undergone an extensive revision. Important revisions have been made upon varied subjects, hyperparathyroidism, hypoglycemia, diabetic treatment, the replacement therapy of ovarian disorders, and studies on the pituitary.

The book contains, in addition to an introductory chapter, discussions on: The Thyroid Gland and Iodine Metabolism, The Parathyroid Glands, The Islets of Langerhans and Insulin, The Adrenal Glands, The Endocrine Secretions of the Organs Concerned With Reproduction, The Pituitary Gland, Some Active and Presumptive Endocrine Principles, and finally a discussion on Endocrine Interrelationships and Antagonisms.

The general utility of the book is enhanced by a good index.

This volume can be commended to the practitioner in search of practical and usable information.

\* *Handbook of Anesthetics*. By J. Stuart Ross, Late Lecturer on Practical Anesthetics, and H. P. Fairlie, Anesthetist to the Western Infirmary, Glasgow.  
 † *Recent Advances in Endocrinology*. By A. T. Cameron, M.A., D.Sc. Professor of Biochemistry, University of Manitoba. Cloth, ed. 2. pp. 406, 55 figures. P. Blakiston’s Son & Co., Philadelphia, Pa.

# REVIEWS

Books and Monographs for Review should be sent direct to the Editor,  
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

## The Biochemistry of Medicine\*

THAT a second edition should become necessary in two years bears out the prediction made in a review of the first edition that this book would be favorably received by the practitioner as well as by workers in special fields.

The present volume represents the changes necessary to bring the text in line with the results of newer studies.

The section on endocrines has been rewritten and now includes an account of anti-endocrine compounds. Changes are found also in the section on vitamins, and, in fact, practically all the various chapters have been revised and often extended. New additions include Von Gierke's glycogen disease, diseases related to disturbances of lipid storage, dinitrophenol in obesity, glycine treatment of myasthenia gravis, and the pentose-nucleotide treatment of agranulocytosis.

## Diseases of the Mouth and Their Treatment†

THAT the mouth and its contents present varied changes in disease, changes sometimes of differential value and even of pathognomonic import, has long been recognized, while its importance as a source of focal infection has been widely discussed in medical literature. In view of these facts the present volume by Prinz and Greenbaum should be of interest to practically all who are engaged in the study of disease.

The specific object of this book is stated in the preface: to gather the enormous mass of scattered information on the subject and present it in an orderly and systematic manner to the medical and dental professions. That this task has been carefully and comprehensively carried out the table of contents bears witness.

As a reference this book should prove of great value to the allied professions of medicine and dentistry as suggesting the importance of a careful survey of the mouth, not only in the presence of oral disease but of disease in general.

The section on gingivitis should be of great value for while this is, perhaps, the most commonly encountered disease in the mouth, it is nevertheless one concerning which the general information is most likely to be confused.

The reviewer, therefore, welcomes its clean-cut and logical discussion in this book. Concerning the constant and varied bacterial flora of the mouth even in health, and recognizing that most of these organisms are peculiarly opportunists, it is apparent that, whatever the primary cause of gingivitis may be, the clinical and laboratory picture is almost immediately complicated, and not infrequently to no small extent determined, by the secondary bacterial invasion.

The borderline between one kind of gingivitis and another is hence often nebulous. Particularly is it refreshing to see that the gingivitis characterized by a predominance of Vincent's organisms, the "trench mouth" of the laity, is clearly stated to be a condition subject to *arrest* rather than cure. For those familiar with its bacteriology have long realized that successive attacks are not new events but represent a recurrence of the original disease.

It is to be expected, of course, that the first edition of any book of this kind is subject to minor criticisms.

\*The Biochemistry of Medicine. By A. T. Cameron, Professor of Biochemistry, University of Manitoba, and Professor of Medicine, University of Manitoba, Ed. 2, Cloth, pp. 518, 31 illustrations. W. B. Saunders Co., Baltimore.

†Diseases of the Mouth and Their Treatment. By Herman Prinz, Professor of Materia Medica and Therapeutics, School of Dentistry, University of Pennsylvania, and Sigmund S. Greenbaum, Associate Professor of Dermatology and Syphilology, Graduate School of Medicine, University of Pennsylvania. Cloth, 602 pages, 287 engravings, 11 colored plates. Lea and Febiger, Philadelphia, Pa.

the Chair of Physiology at the University of Toronto. He remained with this University until 1928, at which time he accepted the Chair of Physiology at Aberdeen University, succeeding Prof. J. A. MacWilliam, who had resigned. In 1922, while connected with the University of Toronto, his collaboration and helpful enthusiasm brought success to the labors of Banting and Best, who succeeded in producing from the pancreas a secretion which they named "insulin," and proved its therapeutic value in the treatment of diabetes. For this discovery these three won immediate recognition throughout the scientific world, and Macleod and Banting were awarded the Nobel prize in medicine for 1923.

It was my good fortune to have been closely associated with Macleod for nearly twenty years. Through a chance meeting with his assistant, Roy G. Pearce, in February, 1915, this acquaintance began, and from it ultimately came Macleod's important contribution to physiology, *Physiology and Biochemistry in Modern Medicine*, the first edition published in 1918. His enthusiasm and clear thinking impressed every one with whom he came in contact. His was the genuine scientific mind. Statements and beliefs meant nothing to him. Only positive proof—so positive that nothing but truth remained—was acceptable. In meditating over his life since his passing, I am reminded very much of Livingston. Both Scotch, both much alike in physical appearance, both with a profound courage and consuming enthusiasm, both sons of clergymen, both working at the end of life under great physical handicaps but without a murmur or complaint. All during the summer of 1934, Macleod read galley and page proof on the seventh edition of *Physiology in Modern Medicine*, and never in any way did he betray that he was ill. Less than a month before his passing I had a long letter telling me of his satisfaction with this, his last edition, and of his hopes for future editions.

Early in my contact with him he expressed the feeling that physiology was of value only in its relationship to clinical medicine and surgery. He taught physiology with this constantly in mind; a viewpoint that is accepted and proclaimed everywhere today.

When the *Journal of Laboratory and Clinical Medicine* was started in 1915, Macleod was a member of the Advisory Editorial Board. With him were Roger S. Morris, Paul G. Woolley, Roy G. Pearce, Dennis E. Jackson, F. P. Gay, Hans Zinsser. Victor C. Vaughan was Editor-in-Chief. Now only Jackson remains of this original group. Gay, Zinsser and Pearce resigned after a few years; Vaughan, Morris, Woolley, Macleod are gone. It was through the pages of this JOURNAL that the discovery of insulin was first published in a medical journal.

It has been the good fortune of few men in medicine to bestow upon mankind a gift so great and so lasting as was bestowed upon it by Macleod in the help he rendered in the discovery of insulin. Vaughan, in commenting on this to me soon after its proof was definite, ranked it of equal importance with the work of Jenner, Pasteur, von Behring and Koch. In fact, he pronounced it of equal value with any discovery ever made in medicine, and so it has proved. Few indeed are the men in medicine who live to see the great work they do bless and benefit mankind. It was Macleod's good fortune to see this, and

# The Journal of Laboratory and Clinical Medicine

VOL. 20

JUNE, 1935

No. 9

Editor: WARREN T. VAUGHAN, M.D.

Richmond, Va.

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## EDITORIALS

John James Rickard Macleod

**M**ACLEOD, the eminent physiologist, co-discoverer of insulin, passed away at his home in Aberdeen, Scotland, Saturday, March 16. For more than two years he had been afflicted with an arthritic infection, but kept on with his labors—teaching and writing—betraying by neither word nor sign of any kind that he believed the end to be near. He carried with him to the last the tradition of his race—to do one's best under all conditions and never complain.

Macleod was born at the mause in Cluny, near Dunkeld, Scotland, September 6, 1876, the son of the late Rev. Robert Macleod. He was graduated from Marisheal College, University of Aberdeen, with the degrees of M.B., Ch.B., in 1898, and four years later obtained the degree of D.P.H. from Cambridge University. About ten years ago the honorary degree of LL.D. was bestowed upon him by University of Aberdeen. In 1903, he was made Professor of Physiology at Western Reserve University, and held this chair until 1918 when he accepted

strength of the patients but also in their gains in weight and improved tonus of their muscles.

Beneficial results have been substantiated in muscular dystrophy by Kostakow and Slauek, Chamutin, Britt and Royster, Beard and Tripoli, although it would appear that the treatment of muscular dystrophy has not been successful in many cases and certain cases do not respond at all.

No ill effects from the use of glycine have been noted. Kidney function even after long periods of glycine administration as measured by urea clearance has remained unchanged.

Glycine which is the simplest of the series of amino-acids is known to be synthesized by the body. Glycine is present in small amounts in many foods; gelatin, however, is the only common substance that contains large amounts of it. It is not considered as an essential amino-acid, in other words animals can live without any in their food, probably because the body under normal conditions can make all it needs. As a result little attention has been given to the possible necessity or even the advisability of administration of glycine.

Glycine should be considered not as a drug but rather a food, in fact as one of the building stones of tissue. At present not much of anything is known of the actual chemical processes involved in the intermediary metabolism. It is possible that even though our bodies synthesize glycine, under certain abnormal conditions the synthesis may be inadequate to prevent muscular fatigability.

Recent investigations of the use of this amino-acid treatment have aroused great interest and have opened a new chapter in the field of metabolism. Glycine offers hope in the treatment of certain myopathies, adds metabolic evidence of the differences existing among these various muscle diseases, and creates a new viewpoint to attempt to elucidate the function and importance of creatine and creatine precursors in muscle physiology.

Early mild stages of myasthenia gravis may be frequently overlooked and mistaken for chronic nervous exhaustion. A diagnosis may be given of functional, neurotic or hysterical and the true condition recognized only after the progress of the disease has developed definite objective signs. The clinical study of patients whose main complaint is fatigability requires in a differential diagnostic effort the consideration of the possibility that these symptoms may be indicative of an early or mild transient form of myasthenia gravis. Oppenheim's original description of the cardinal symptoms of myasthenia gravis, namely weakness and fatigability of groups of muscles, especially those innervated by cranial nerves (bulbar palsy, ptosis, ophthalmoplegia) should be borne in mind.

However, a word of caution should be given in the application of glycine to various fatigabilities of patients without pathologic changes in muscle. Although it will be of interest to observe the effects of glycine in simple fatigability great care in the subjective interpretation of symptomatic results will be necessary.

The studies and recent clinical investigations of the use of glycine are indeed very enlightening and open a new field of metabolic study. Glycine, however, will probably not prove to be a panacea for all disturbances of muscles.

seeing it, no doubt, made death easier for him. With one's work done, especially a work so beneficial as that done by Macleod, one can face the end just as one lies down at the close of the day to sleep peacefully, knowing that he has done his best.

His students caught the master's vision and enthusiasm. He was never so happy as when he had a group around him, demonstrating, proving, inspiring. To these he has passed the torch, and in them will his life and his work live on, while the millions now and in the future who live because of insulin bless and revere his name.

—C. V. Mosby.

### Glycine, Glycocoll, or Amino-Acetic Acid

GIBSON and Martin and Brand, Harris, Sandberg and Ringer, in their investigations of the origin of creatine, were among the first to recognize the importance of glycine. The significant relationship between glycine,\* a substance that the normal person can readily synthesize, and creatine, which has been demonstrated to play an important part in muscle function, suggested to several investigators that prolonged administration of the amino-acid might influence the myopathies. The discovery of metabolic functions of creatine in muscle metabolism has attracted considerable clinical interest in the study and treatment of disorders of the muscular system. Evidence is gradually accumulating which indicates that the metabolism of creatine has a wider clinical importance than in the myopathies alone, for changes in creatine excretion have been noted in individuals not suffering from disorders of the muscular system.

Karl Thomas, one of the most noted of German physiologic chemists, has been particularly interested in all problems connected with metabolism. From his laboratory in Leipzig came a report of the beneficial effect of the administration of glycine to patients with progressive muscular dystrophy. This was a rather striking confirmation of the hypothesis that glycine might influence the myopathies.

Following this work of Thomas, Milhorat and Technor, Boothby, in conjunction with Wilder, Hench and the section of neurology, of the Mayo Clinic, not only studied the use of glycine in muscular dystrophy but also extended the investigation to include patients with undoubted myasthenia gravis and even cases of variations of fatigability unassociated with any type of muscular pathologic changes.

Since these early reports Remen, Schnitt, Van Dalsem, Reese, Taylor, Beard, Tripoli, and others have used glycine in cases of myasthenia gravis and feel confident that glycine as a therapeutic agent in this condition is of significant value. Although in a very few of the most severe cases benefit from it has not been obtained, unmistakable benefit has been obtained in other severe cases and in all of the milder cases. The improvement obtained is not only in the increased

\*Glycine must not be confused with the photographic developer sold under the trade name, glycin, which is an entirely different substance and is distinctly poisonous.

## CORRESPONDENCE

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To the Editor:

IN REGARD to Dr. Kracke's letter in your journal for January, in which he criticized my article on the rôle of amidopyrine in the etiology of granulocytopenia, I feel that several of his criticisms are entirely unwarranted, and contrary to the consensus of scientific opinion.

Dr. Kracke states that amidopyrine is prepared from phenyl hydrazine and comes ultimately from benzene and anilin, and goes on to state "it is not prepared from pyrazolon." Quite true, but can he deny that, regardless of its derivation, it contains a pyrazolon ring?

Dr. Kracke states that amidopyrine does not have an imido radicle, but that it has an amine radicle instead. In this statement he is absolutely wrong, as shown by McGuigan, who in *A Textbook of Pharmacology and Therapeutics* (Saunders, 1928), page 137, states: "The antipyretic action apparently resides in the benzene ring. Benzol itself is not antipyretic because it cannot enter into reaction with the body tissues, but phenol has some antipyretic action, anilin and phenyl hydrazine act more strongly. Paraminophenol is also antipyretic. The latter is more antipyretic and causes less blood destruction than anilin or phenyl hydrazine. In antipyrene, the pyrazole ring or complex apparently is also antipyretic since the antipyretic properties are absent in 1-phenyl 3-methyl pyrazolon, and only when the imido hydrogen is replaced by methyl does it appear." Naturally what is true of antipyrene, is equally true of its derivative, amidopyrine.

In *An Introduction to Chemical Pharmacology* (Blakiston, 1921), McGuigan classes antipyrene as an artificial alkaloid, and states that like alkaloids, it unites with acids, and when prepared, it is combined with HI. In his list of alkaloids, on page 224, he includes antipyrene.

Kracke states that there is insufficient evidence to ban amidopyrine. Yet, in his article on "The Etiology of Granulopenia" (*American Journal of Clinical Pathology* 4: 453, 1934), he states: "first, that granulopenia, based on a review of 1,385 death reports, is a disease that affects nurses, physicians and medical people more than any group in the country; second, that certain drugs of the benzene ring class are capable of being oxidized to toxic products which are capable of producing granulopenia in rabbits." Further in the same article, he states: "It seems that there is constantly increasing evidence that acute fulminant granulopenia and probably the chronic types as well are caused for the most part by the administration of easily oxidizable benzene ring drugs. In the literature there now have accumulated reports of fifty cases in which these drugs are incriminated; notably amidopyrine, occasionally phenacetine and in one instance dinitrophenol." Furthermore, the Food and Drug Division of the U. S. Department of Agriculture has recently sent out a bulletin warning physicians about two dangerous drugs—amidopyrine and cinchophen. The burden of proof is now upon the champions of amidopyrine.

When Kracke classed amidopyrine as a benzene ring compound, I saw fit to disagree with him in my original article. I still maintain that it is a pyrazolon compound, and that the benzene is a side chain. I have already quoted McGuigan in classifying a pyrazolon compound, antipyrene, as an artificial alkaloid. Evidently, he regards it as entirely different in structure from acetanilid and phenacetin, both of which are mentioned in his book, but neither of which is considered an alkaloid. Furthermore, Beckman in *Treatment in General Practice* (Saunders, 1934), under "Agranulocytosis—Therapy" gives the heading: "The Withholding of Drugs Containing Pyrazolon Derivatives," and mentions specifically Madison and Squier's 14 cases, stating that all of these gave a history of having taken amidopyrine. Evidently, he recognizes amidopyrine as a pyrazolon compound.

As to the safety of acetanilid, which Kracke seems to doubt, in Kracke's own article, previously quoted, on page 455, he states: "We reported one patient who took large amounts of acetanilid. However, this patient has since been reinvestigated and was found to have



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—C. J. B.

# *The Journal of Laboratory and Clinical Medicine*

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VOL. 20

JULY, 1935

No. 10

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## CLINICAL AND EXPERIMENTAL

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### THE INFLUENCE OF CLAUDE BERNARD'S EXPERIMENTAL METHODS ON MEDICINE\*

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JOSEPH L. MILLER, M.D., CHICAGO, ILL.

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CLAUDE BERNARD was born of humble parents at St. Julien, France, in 1813. As a boy, he apparently showed evidence of unusual ability as the curé taught him Latin. Later he was sent to a Jesuit school at Villyfranche. After completing his studies here, he went to college at Lyons, but remained only a short time. No satisfactory explanation has been given for his early discontinuance of college studies. He then entered a pharmacy in Lyons where he was taught the art of dispensing. He spent his free evenings at the theater and during this period wrote a one-act comedy, "The Rose of the Rhone," which met with considerable success. Encouraged by this, he wrote a five-act tragedy which was not a success. Apparently thinking he might have a larger field in Paris, he took his manuscript and a letter of introduction to the literary critic at the Sorbonne. The critic recognized that Bernard had considerable ability, but advised him that, inasmuch as he had worked in a pharmacy, he should study medicine and devote his spare time to literature.

In accordance with this advice, Bernard entered the Medical School of the College of France and supported himself by tutoring. Of the preclinical sciences, he was especially interested in physiology and anatomy. In dissection he became very expert as he was gifted with an unusual amount of manual dexterity. This proved to be an important factor in his later success in experimental physiology. His teachers considered him indifferent to, and indolent in, all subjects with the single exception of anatomy.

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\*Presidential Address Delivered before the Institute of Medicine of Chicago, December 4, 1931.

Received for publication, December 5, 1931.

received amidopyrine also. We believe, therefore, that acetanilid can be ruled out as an etiologic drug since there is no report of any case of granulopenia following the use of that drug alone."

As to the advisability of combining acetanilid with alkalies and caffeine, Worth Hale demonstrated that alkalies decreased the toxicity of acetanilid by 20 to 25 per cent, but thought that caffeine increased the toxicity. McGuigan, during the past year, exploded this theory in regard to caffeine, and by carefully conducted experiments, showed that caffeine reduced the toxicity of acetanilid. Furthermore, the U.S.P. preparation, *pulvis acetanilid compound*, consists of acetanilid, caffeine, and sodium bicarbonate in the proportions of 100, 15, and 30.

The *J. A. M. A.* under the editorial column of *Therapeutics* (104: 1229, 1935), under "Analgesia," recommends aspirin for the milder types of pain, and states that if it does not suffice to relieve the pain, acetanilid may be added to it. It also refers to specific indications for phenobarbital, morphine or codeine. It ignores amidopyrine.

I still maintain that the coal-tar analgesics should be placed into three distinct groups: (1) acetanilid and phenacetin, derivatives of anilin, and excreted as paraminophenol; (2) antipyrine and amidopyrine, pyrazolon compounds, excreted largely unchanged; and (3) the salicylates, excreted as ethereal sulphates and glucuronates. I do not regard it as chemically or medically correct to include groups (1) and (2) together as "benzamide compounds" or benzene ring derivatives, as I consider the pyrazolon ring important in differentiating the two, and I consider that they comprise two distinct groups. I also maintain that amidopyrine has been our worst offender as the causative factor of granulocytopenia, and we should recognize this fact, and properly classify the drug as a pyrazolon compound, and not obscure the issue by speaking of "benzamine drugs," and "benzene ring drugs," where the benzene ring may have no bearing upon the case, as the other benzene ring drugs do not have the disastrous effects of amidopyrine. Those who adhere to the "benzene ring" theory will have to produce greater evidence than they have thus far produced, to give credence to their theory. Meanwhile, I shall adhere to the hypothesis which clinical evidence seems to produce—amidopyrine is distinctly different from other coal-tar analgesics; and in my opinion, the pyrazolon group, and more specifically the imido radicle, is responsible for its toxic properties.

Very truly yours,

(Signed) LUCIUS FELIX HERZ, PH.B., M.D.

145 W. 71ST STREET, NEW YORK

MAY 2, 1935.

In France the views of Cuvier, the morphologist who died in 1832, still dominated physiology. He was a through and through vitalist and consequently scoffed at experimental methods as a means of acquiring information. Bernard's chief, Magendie, was not willing to admit that vitalism was non-existent, but he believed much could be learned from experimental studies. Michael Foster referred to him as "the apostle of experimental methods in France." He experimented, however, without previous premeditation, believing that a preconceived idea was not essential (and might be a handicap). According to Foster, "He thrust his knife here and there to see what would come out." Magendie said of himself, "I am a mere street scavenger of science. With my hook in my hand and my basket on my back, I go about the streets of science collecting what I find." (Quoted by Michael Foster in "Claude Bernard," *Masters of Medicine Series*.) However, he made many valuable contributions to science and in addition gave a great impetus to the experimental method of approach. No doubt he had a great influence on Claude Bernard.

Bernard, therefore, was beginning his life's work at a time when vitalism, which had been such a handicap to investigation, was rapidly on the wane. He had the wisdom to strike out on new lines, by combining premeditation with experimentation, and throwing vitalism into the discard. His opinion of the medicine of that day is expressed in his opening lecture to students in 1847: "Scientific medicine, gentlemen, which it ought to be my duty to teach, does not exist." Shortly after this a special chair of physiology was established for him at the Sorbonne and in 1855, after Magendie's death, he was given the latter's chair at the College of France. In 1868 he was given the highest honor that could be bestowed on a scientist, a membership in the French Academy.

In 1865 his book entitled *Introduction to the Study of Experimental Medicine* was published. This book was translated into English in 1927. This paper is largely in the form of a review of this book. Henry E. Sigerist in his book *The Great Doctor* says of this volume: "It is the fate of medical books to age quickly, but there are exceptions. If in contemporary Paris we examine the bookseller's windows in the neighborhood of the medical college, we shall find exposed for sale a work whose first edition was published in 1865." This is Claude Bernard's *Introduction to the Study of Experimental Medicine*. Sigerist refers to a statement made by Pasteur one year after this book was issued: "Never has anything clearer or more complete, more profound, been written about the true principles of the difficult art of experiment."

In his opening discussion he says that information acquired by medicine up to the present time has been through various evanescent systems, all of them empirical. He states that medicine is turning toward its permanent scientific path. Systems are being abandoned and medicine is becoming more analytical and gradually will become an experimental science. It is his opinion that the approach to this will come through applying the experimental method to physiology, to pathology, and to medicine. He continues by saying that, during the period of empiricism, physiology and medicine could advance separately, "but now they must mutually support each other with physiology as the basic science." "I think that medicine is destined to be an experimental

During his fifth year he was appointed an interne and, by a happy chance, was assigned to Magendie who at this time was one of the physicians at Hotel Dieu. Magendie observed Bernard preparing an animal for experimental demonstration and was impressed by the latter's remarkable skill in dissection. He immediately appointed him his assistant in the preparation of experimental demonstrations. At this time Bernard was transferred to the College of France where Magendie held a chair.

Claude Bernard took his degree in 1843, at the age of thirty years. His thesis was on gastric juice and the part it plays in digestion.

As assistant to Magendie, he worked under the most unfavorable conditions. He had a small gloomy room in the college which served as his experimental laboratory. A small group of friends met with him on Monday evenings to discuss his experiments. The antivivisectionists were very active at this time and were responsible for many of his worries.

The following is a list of his most important discoveries:

He ascertained the method of action of the various digestive juices.

He demonstrated the rôle of the pancreas in the digestion of fats.

He showed that sugar was always present in the blood, and that it was stored in the liver and muscles in the form of glycogen.

He pointed out that when a certain spot in the medulla was punctured by a needle there followed a great increase in the blood sugar and glycosuria. This experiment was followed up to determine why this occurred, and he came to the conclusion that it was due to stimulation of sympathetic nerves.

To him belongs the credit for first demonstrating that the sympathetic nervous system was concerned with metabolism. Following this, he investigated the rôle of the sympathetic nervous system on the various organs.

He demonstrated that curare acted on the terminal ends of the motor nerves. He then investigated other poisons and found that each attacked a particular tissue. He showed that carbon monoxide entered into combination with hemoglobin. He then investigated a number of drugs and found that, like the poisons, they acted on certain tissues. He thus extended his field of experimental physiology into the domain of experimental pharmacology.

In order to appreciate Claude Bernard's influence on medicine, we should understand the condition of physiology during the first half of the nineteenth century. Vitalism, a child of Galen, and at this time the last remnant of theological influence on science, still controlled, or at least interfered with, efforts to advance knowledge of living matter by experimental means. Vitalism was a theory (or perhaps more accurately, a creed) that there was a vital process in living matter which did not admit of explanation by mechanical or material means.

Johannes Müller, known as the father of scientific medicine in Germany, was the outstanding physiologist in his homeland at this period. In his earlier years he had made some physiologic investigations on the special senses. He was also a biologist and morphologist. He clung to vitalism, but not to the extent that he thought it impossible to explain some of nature's secrets by experimental means. He did much to encourage investigation.

In England physiology, as a profession, had few followers, but these few workers were following experimental methods.

verified, we at least have collected some facts that are useful to science. "The tending to a preconceived idea always has been and always will be the first flight of an investigative mind."

Bernard was not only a scientist, but also a philosopher as shown by the following quotation: "The experimental method is not a natural tendency for man. Only after lengthy wandering in theological and scholastic discussion has he recognized at last the sterility of his efforts in this direction." "The experimental mind differs from the scholastic chiefly in its modesty because his experiments make him moment by moment conscious of both his relative and absolute ignorance."

Again philosophizing, he says that the human mind in the course of evolution has passed first from feeling to reasoning and finally to the experimental method. First, feeling alone imposed itself on reason. This created the faith in theology. "Reason or philosophy, the mind's next mistress, brought to birth scholasticism." "Experimentation in natural phenomena brought forth truths concealed from feeling or reason. In the search for truth, however, feeling takes the lead: it begets the a priori idea, reason develops this idea. In turn reason must be guided by experiment." "The experimental method is concerned only in objective truths."

"Experimental ideas do not arise spontaneously. Usually such ideas arise as a result of observation." In his work he relates that not infrequently in the course of an experiment he made an observation that had nothing to do with the subject under investigation, but which furnished a lead for further experimentation.

He refers to a statement frequently made, that to make discoveries one must be ignorant. This is in line with a statement made by Sir Clifford Allbutt in regard to a famous surgeon of the sixteenth century: "He was uneducated and hence did not have the handicap of a speculative intellect." Bernard says that this statement contains a truth as uncontrolled theories lead one astray, especially if unable to ignore truths that are in conflict with one's theory. "We never make experiments to confirm our ideas but to control them."

He warns against the man who conducts his experiments only for the purpose of refuting a colleague, rather than to collect facts: "At the same time they make poor observers because they choose only those observations that suit their purpose." "A man of science wishing to find truth must keep his mind free and calm and if possible never have his lids bedewed, as Bacon says, by human passions."

If in error we must gladly admit it. "When we make a mistake, the service which we owe to truth requires that we should never fear retraction." "Let me say it is always instructive to acknowledge an error. It is not enough to say 'I was mistaken'; we must say how we were mistaken."

He discusses the conclusions that may be drawn when a positive result is followed by one or more negative ones. "Negative facts, no matter how numerous they may be, never destroy a single positive fact. That is why pure and simple negation is not criticism and this method should be absolutely rejected in science, because science is never built on negation."

and progressive science and precisely because of my conviction in this respect I am putting forth this work with an object of contributing my share toward encouraging the development of scientific and experimental medicine." He discusses the difference between observation and experiment, "the former appears of itself; the latter is the fruit of an effort." He agrees with Cuvier when he says, "The observer listens to nature; the experimenter questions and forces her to unveil herself." The experimenter must have some genius as an inventor in order to devise apparatus. "In the experimental sciences all progress is measured by improvement in the means of investigation." "In scientific investigation minutiae of method are of the highest value. An instrument constructed in some special way may often suffice to solve the most abstract problem." Simple noting of facts is of limited value: "We must reason about what we have observed." An effort should be made to have some guiding lead before beginning an experiment. Occasionally an experiment is performed without any preconceived idea: "The experiment is then an observation induced with the object of bringing to birth an idea."

The experimenter must possess three qualities—he must have ideas; must be an unbiased observer; and must have manual dexterity. Bernard's early work in anatomy had trained his hand and eye. "Observers simply note the phenomena before their eyes." "Observers then must be photographers of phenomena. Their observations must accurately represent nature." "We must observe without any preconceived ideas, the mind must be passive, that is, it must hold its peace. It listens to nature and writes at her dictation."

When the experiment is begun "The experimenter must now disappear, or rather change himself instantly into an observer, and it is only after he has noted the results of his experiment exactly that his mind will come back to reason, compare, and decide whether his experimental hypothesis is verified or disproved by these results." He proceeds further along this same line: "I may say that our experimenter puts questions to nature, but as she speaks he must hold his peace, he must note her answers, hear her out, and in every case accept her decisions." "Put off your imagination, as you take off your overcoat, when you enter the laboratory, but put it on again as you do your overcoat when you leave the laboratory. Before the experiment and between whiles, let your imagination wrap you around; put it right away from you during the experiment itself lest it hinder your observing power." Bernard emphasizes this saying by another: "He must never speak for her nor listen partially to her answers by taking from the results of an experiment only those things that support or confirm his hypothesis."

An experiment is made up of two parts—premeditation on the method with which the experiment is to be carried out; and notation of the result. "I consider it, therefore, an absolute principle that experiments must always be devised in view of a preconceived idea, no matter if the idea be not clear nor very well defined." To those who condemn the use of a hypothesis or preconceived idea, he answers by saying, "They confuse the method of making the experiment with the method of recording the result." "We must give free reign to our imagination, but we must regulate it." If the hypothesis is not

He refers to a statement made by Laplace, when asked why he wished to admit physicians to the Academy of Science (as medicine was not a science), "To get them among scientific men."

Referring to the origin of therapeutics: "The first tendency of man was to help his neighbor in distress. The agent employed might be a drug, or moral or religious advice." "But after this first flight of fancy which started so to speak from the heart, men have been led to reflect and, seeing the sick recover often with and without medicine, they were inclined to ask whether medicine was useful or harmful."

"Experimental medicine, unlike other cults, has no system and rejects nothing that appears reasonable, but maintains an analytical attitude toward medicine." "The scientist is not satisfied with the statement that quinine cures fever; he wishes to know the cause of the fever and the means of cure."

He says that the scientific physician is always more perplexed at the bedside than the empiricist, because he realizes the limitation of drug therapy. "He does not object to using empirical remedies but does insist on observing their effect and drawing conclusions." "Empiricism is nothing but the first step of the experimental method." "We must suffer empiricism but not accept it as a system." "Experimental medicine is only a union of expectancy and empiricism, enlightened by reason and experiment." "Medicine is destined to get away from empiricism." "Of course we shall not see scientific medicine blossoming in our day but that is not man's lot; those who sow and laboriously till the field of science are not also destined to reap the harvest." "We must remember that the one unchanging scientific principle in medicine is the absolute determinism of phenomena."

He warns against words that mean nothing, yet at first thought might appear to explain. "We must constantly be on our guard against the traps which our minds perpetually set for themselves."

He believes the teacher should simply show the student the goal: "He should then leave him free to move about and in his own way according to his own nature, only coming to his aid if he sees he is straying."

"A desire for knowledge is the moving influence in the investigator." "Those who do not know the torment of the unknown cannot have the joy of discovery which is certainly the liveliest that the mind of man can feel."

Claude Bernard's faith that advancement in medicine would, in a large measure, be due to discoveries made through experimental physiology was based largely upon the epoch-making discoveries made in his laboratory. He no doubt felt this was only a beginning effort directed toward making medicine a science. "Hence the prudent and reasonable course at the present moment is to explain all that part of disease which can be explained by physiology, and to leave that we cannot so explain to be explained by the future progress of biologic science."

The science of bacteriology at this time was unborn. However, the application of this science to medicine largely came through experimental physi-



To Bernard must be given the credit for recognizing the close relationship between physiology and clinical medicine. At the present time this relationship is not so widely grasped as it should be. In truth the symptoms of disease are merely the manifestations of a disturbed or pathologic physiology. We might add that the signs of disease are the expression of pathologic anatomy which in turn is responsible for the disturbed physiology.

Bernard in the third chapter of the third part of his book discusses the application of physiologic experimental methods to clinical medicine. He states repeatedly that scientific medicine will develop through a knowledge of physiology. He says that the beginning of all science is empiricism, based on a chance observation or experience. Empiricism can never be a permanent state of science, but it is the first preliminary step. While empiricism largely dominated medicine in his day, his faith was unbounded that eventually empiricism would be replaced by science through the experimental method. "The physician's new observations are generally the result of chance; that is, the patient comes to the physician and this act may be considered chance." "In such circumstances the physician's originality lies in his ability to see the fact that chance has presented to him and in not letting it escape and his only merit is accurate observation." His pride in the importance of physiology is expressed as follows: "To make good medical observation it is not only necessary to have a good observing mind, but also to be a good physiologist." "We must be, as it were, a photographer of Nature."

"All early therapeutic observations were matters of chance, the observing of the effect of certain remedies, but control experiments are necessary before drawing conclusions." He recommends the method of Pinel who said in his clinic: "This year we will observe diseases without treating them; next year we will treat them." "Only by this method are we enabled to know the course of untreated disease." "Therapeutic investigation conforms to exactly the same rules as physiological and pathological investigations." "The method of observation and experiment is still the same, unchangeable in its principles and offering only a few peculiarities in its application."

He states positively that scientific medicine does not mean that bedside observation can be dispensed with: "Quite the contrary, it is the observing physician who must be depended upon to draw conclusions." "It is essential, however, that the observer have a scientific mind; that is, he must love truth for science's sake." He criticizes those who believe that clinical medicine is largely an intuitive science—"Such physicians deny science and encourage laziness, ignorance and charlatanism." He admits that experience may be a valuable aid to the physician, largely due to accumulation of empirical knowledge, "But what I blame is wilfully staying in this empirical state and not trying to get out of it." He also criticizes those who say that medicine is largely an art: "There is no such thing as a medical artist. Physicians calling themselves such, injure medical science because they exalt a physician's personality by lowering the importance of science." He believes the best method of advancing scientific medicine is to train the medical student in experimental methods.

started with 5 units thirty minutes before each of the three meals. At intervals of several days as indicated by the blood sugar two hours after breakfast, the insulin dosage was increased a few units at a time until one patient was receiving as much as 75 units daily. All of the patients in this series were on a general diet with one exception, Case I. N., who was on a high carbohydrate diet. Blood sugar and qualitative urine sugar and acetone determinations were made daily in most instances. After the insulin dosage had been increased to 36 to 75 units daily and maintained at this level for several days, the insulin injections were suddenly discontinued. Blood sugar and urine sugar determinations were made after each meal until two-hour postabsorptive blood sugar level had returned to normal (pre-insulin level), and the glycosuria had disappeared. Glucose tolerance tests were observed in two cases at the time of discontinuation of insulin, and in one other case both during the course of insulinization and at intervals after its interruption. When tolerance tests were performed during a period of insulinization, insulin and breakfast were omitted on the morning of the test, the patient was given 50 gm. of glucose by mouth, and then the regular dosage of insulin was resumed with the noon meal.

#### SUMMARY OF RESULTS

The detailed account of these experiments is given in the accompanying case reports and tables.

During the periods of insulinization when insulin was given in progressively increasing amounts to as high as 75 units daily, blood sugars two hours after breakfast were usually normal, sometimes somewhat elevated. There was a tendency, however, in some of the subjects for the afternoon blood sugar to be somewhat low; in such cases a malted milk was allowed in the afternoon in addition to the usual diet. Hypoglycemic symptoms were rarely observed. Only one hypoglycemic shock was encountered, Case E. L., following vomiting of his supper; this, however, was promptly relieved by an intravenous injection of glucose.

After the first meal following discontinuation of the insulin injections, a certain degree of hyperglycemia was uniformly observed, the blood sugar rising as high as 250 to 300 mg. in some instances. Four of the six patients showed some glycosuria. Readjustment to the pre-insulin blood sugar level was prompt in some cases requiring only one or two days; in other subjects as long as three days or more were required before the hyperglycemia subsided and the glycosuria disappeared. Glucose tolerances in three cases were diminished at the time the insulin injections were interrupted. One of these, Case H. J., showed a progressive diminution of glucose tolerance with increasing amounts of insulin; at the time insulin was discontinued when the patient was receiving insulin units 25 t. i. d. or 75 units daily, the curve was of the mildly diabetic type, reaching a maximum of 253 mg. at the end of 1.5 hours. The return of the tolerance test to normal in this patient was slow; even after eight days it had not returned to the original level.

In connection with a study of the respiratory quotients, four other patients have been given insulin in doses varying from 33 to 81 units daily. All

ology. If we review the advances made in internal medicine through the past decades, we are impressed with the important rôle played by experimental physiology.

Bernard's discoveries have stood the test of time, the best evidence of the soundness of his method. I know of no more appropriate adornment for the walls of a research laboratory than some of the mottoes of this famous physiologist.

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## A STUDY OF THE RÔLE OF INSULIN IN METABOLISM IN NONDIABETIC PATIENTS\*

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### I. TRANSITORY HYPERGLYCEMIA AND GLYCOSURIA FOLLOWING DISCONTINUATION OF INSULIN

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B. B. CLARK, PH.D., R. B. GIBSON, PH.D., AND W. D. PAUL, M.D.,  
IOWA CITY, IOWA

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THE administration of insulin to nondiabetic patients in an attempt to increase body weight dates back to shortly after the discovery of insulin by Banting and Best in 1922. Pitfield<sup>1</sup> in 1923 reported considerable success with its use in infantile inanition, and Falta<sup>2</sup> in 1925 reported uniform results in malnutrition in adults. Since then some 200, or more, reports have appeared; that of Metz<sup>3</sup> has been one of the most enthusiastic. Generally most of the cases reported have shown some gain in weight. However, in this large number of publications data aside from the essentially "clinical" are strikingly lacking, even blood sugars. The administration of insulin to nondiabetic subjects has apparently been regarded as a physiologic procedure presumably not associated with any evidence of a disturbance of metabolism or "deleterious effects."

This laboratory became interested in this subject upon observing a rise in the blood sugar following discontinuation of insulin given to an anorexia case, which suggested that a temporary hyperglycemia and even a glycosuria might be induced in this way in nondiabetic subjects. Data supporting this suggestion obtained from blood sugars and glucose tolerance tests in a number of patients during and following a period of insulinization have been presented in a preliminary report (Paul, Clark, and Gibson 1932).<sup>4</sup> These experiments are reported in this paper in detail. Independently Wilder, Smith, and Sandiford<sup>5</sup> observed a decrease in glucose tolerance in two obese patients in whom they were studying the effect of insulin upon the rate of weight loss.

#### METHOD

Usually after a period of hospitalization, during which blood sugars,<sup>6</sup> 50 gm. glucose tolerance tests,<sup>7</sup> and body weight were observed, insulin was

\*From the Laboratory of Pathological Chemistry, and the Department of Internal Medicine, the State University of Iowa.

Received for publication, December 15, 1934.

TABLE II  
E. L. HOSPITAL, No. G3821

DATE	BLOOD SUGAR	INSULIN BEFORE MEALS	BODY WEIGHT	REMARKS
	mg. per cent	units	pounds	
5/ 2/32			115	
5/ 3	100			
5/ 4	133 (2 P.M.)	5- 5- 5		
5/ 5	44 (2 P.M.)	5- 7- 7		
5/ 6		7- 7- 7		
5/ 7		7- 7- 7	117	
5/ 8	173	7- 7- 7		
5/ 9	50 (2 P.M.)	7- 7- 7		
5/10	30 (2 P.M.)	7- 7- 7	117½	
5/11	100	7- 7- 7		
5/12	189	7-10-10		
5/13	112	10-10-10		
5/14	118	10-10-10	118	
5/15	102	10-10-10		
5/16		10-10-10		
5/17		10-10-12	120	
5/18		12-12-12		
5/19	78 (2 P.M.)	12-12-12		
5/20		12-12-12		
5/21	120	12-12-12	121½	
5/22	28 (8 P.M.)*	12-15-15		
5/23	179	10-12-12		
5/24	259 (2 P.M.)	12- 0- 0	122½	Glycosuria 1.1%
	176 (7 P.M.)			
5/25	167			Glycosuria trace
	145 (2 P.M.)			Glycosuria trace
5/26	159			Glycosuria trace
	104 (2 P.M.)			
5/27	147			

\*Hypoglycemia shock following vomiting of supper. Intravenous glucose was given. Blood sugars were taken at 9 A.M., two hours after breakfast, unless otherwise indicated.

30 or 50 mg. without definite symptoms of hypoglycemia. To obviate this possibility, the patient was allowed a malted milk in the afternoon. On the evening of May 22, he had vomited his supper without reporting it to the nurse. At 8 P.M. he was found in hypoglycemic shock with a blood sugar of 28 mg. A few grains of glucose intravenously promptly relieved the symptoms. Insulin was discontinued on May 24 with the morning injection as the last. The blood sugar two hours after breakfast was 179 mg., but after the noon meal without insulin, the blood sugar was 259 mg. with a glycosuria of 1.1 per cent. The blood sugar at 7 P.M. was 176 mg. with a trace of sugar in the urine. Blood sugars on the two following days were 167 mg. and 159 mg., respectively, with still a trace of sugar in the urine. By the fourth day after discontinuing insulin, the morning blood sugar had dropped to 147 mg. and the urine gave no qualitative test for sugar. The patient gained seven pounds during the period of insulinization.

I. N., male, aged forty-nine years, was admitted to the hospital April 24, 1932. A diagnosis of arteriosclerosis, duodenal ulcer, and incomplete heart block was made. The patient was placed on a high carbohydrate diet. His weight on admission was 123 pounds, but at the beginning of the experiment, May 4, was 120 pounds. Insulinization was started on May 4 with 5 units before meals and increased the following day to 7 units t. i. d. The dosage was later increased to 10, 12, and 15 units before meals on May 12, 17, and 22, respectively. Blood sugars both morning and afternoon were normal or somewhat elevated. Insulin was discontinued with the evening injection on May 24 as the last. The blood sugar at 9 A.M. the following day was 253 mg. with a glycosuria of 0.5 per cent, and at 2 P.M. 179 mg. By the next day the blood sugar level had returned to normal. The patient's

of these subjects showed some degree of hyperglycemia when the insulin was discontinued, and two showed a glycosuria. The glycosuria in one patient persisted for five days.

A gain in weight has been observed during the period of insulinization in all of the patients except two to whom we have given insulin; the gains amounting to from four to eighteen pounds during periods of two to six weeks. These patients were kept on a roughly constant diet, except for an afternoon lunch in two cases; the increase in appetite was not marked.

#### CASE REPORTS

M. G., female, aged fifty-nine years, was admitted to the University Hospital Nov. 19, 1931. Her entrance complaints were insomnia, anorexia, and loss of weight of one year's duration. The physical examination and laboratory findings were normal. The patient's weight on admission was 115 pounds. She was started on insulin November 29 with 5 units thirty minutes before meals; on December 5 the dosage was increased to 10 units before breakfast, and 5 units before lunch and supper. It was observed that the insulin caused little decrease in the two-hour postabsorptive blood sugar level. Insulin was discontinued on December 9 with the evening injection. The blood sugar on the following morning was

TABLE I  
M. G. HOSPITAL NO. F9881

DATE	BLOOD SUGAR	INSULIN BEFORE MEALS	BODY WEIGHT
	mg. per cent	units	pounds
11/25/31	170		
11/29		0-5-5	113
11/30		5-5-5	
12/ 1		5-5-5	119
12/ 2	186	5-5-5	
12/ 3	133*	5-5-5	
12/ 4	159	5-5-5	
12/ 5	118	10-5-5	120
12/ 6	152	10-5-5	
12/ 7	189	10-5-5	
12/ 8	98	10-5-5	122
12/ 9	124	10-5-5	
12/10	226		
12/11	94		
12/12	133		122
12/14	124		

\*Blood sugar taken at 2 P.M. All other blood sugars are at 9 A.M. two hours following breakfast.

226 mg. which was significantly higher than any value previously obtained. The blood sugars on subsequent mornings were only slightly elevated above normal. During the period of insulinization, the patient's weight had increased to 122 pounds, a gain of nine pounds.

E. L., male, aged seventy years, was admitted to the hospital April 27, 1932. His entrance complaint was gastric distress relieved by vomiting. He stated that the vomiting was usually induced by irritating his pharynx. The positive physical findings were marked weight loss and tenderness over lumbar sacral spine. Laboratory findings were normal. On roentgenologic examination, the stomach and duodenum were normal, but osteoarthritic changes were found involving the lumbar sacral spine. The patient's weight was 111 pounds on admission and increased to 115 pounds during the first five days. Insulin was started on the seventh day, May 4, with 5 units before meals, increased to 7 units t. i. d. on May 5, 10 units on May 12, and 12 units on May 17. During this period morning blood sugars were normal, sometimes somewhat elevated; however, some of the afternoon blood sugars were as low as

TABLE V  
E. G. HOSPITAL No. G2413

DATE	BLOOD SUGAR	INSULIN BEFORE MEALS	BODY WEIGHT	REMARKS			
	mg. per cent	units	pounds				
6/ 7/32			108				
6/ 9	92*, 1			*Fasting; tolerance			
6/10		0- 5- 5					
6/11	112 (2 P.M.)	5- 5- 5					
6/12	118 (2 P.M.)	5- 5- 6					
6/13	78 (2 P.M.)	6- 6- 6					
6/14	85 (2 P.M.)	6- 6- 6					
6/15	133 (2 P.M.)	6- 6- 8					
6/16	135 (9 A.M.)	8- 8-10					
6/17	114 (2 P.M.)	10-10-10					
6/18	99 (9 A.M.)	10-10-10					
6/19	116 (9 A.M.)	10-10-12					
6/20	79 (2 P.M.)	12-12-15					
6/21	52 (9 A.M.)	15-15-15					
6/22	90 (2 P.M.)	15-15-15					
6/23	39 (9 A.M.)	15-15-15					
6/24	65 (9 A.M.)	15-15-15					
6/25	162 (9 A.M.)	15-15-18					
6/27	60 (9 A.M.)	18-18-18					
6/28	57 (9 A.M.)	18-18-18	112				
6/29	100 (9 A.M.)	18-18-18					
6/30	69 (9 A.M.)	18-18- 0					
	120 (2 P.M.)						
	295 (7 P.M.)			Glycosuria 0.139			
7/ 1	133*, 2			*Fasting; tolerance			
	159 (7 P.M.)						
7/ 2	138 (9 A.M.)						
7/ 3	124 (9 A.M.)						
7/ 4	179 (9 A.M.)						
7/ 5	129						
7/ 7			114				
*Glucose tolerance: 50 gm. glucose by mouth							
Hours	0	0.5	1	1.5	2	3	Urine
<sup>1</sup> B. S. mg. per cent	92	173	226	208	176		Negative
<sup>2</sup> B. S. mg. per cent	133	200	182	236	241	142	1 hr. 0.54%
							2 hr. 0.22%

weight had increased to 124 pounds at the time insulin was stopped. It is interesting to note that the insulin had no effect on the cardiac lesion and no strikingly favorable effect on his gastric distress.

E. H., female, aged twenty-two years, was admitted to the hospital June 2, 1932. A diagnosis of amebic dysentery and ulcerative colitis was made. The patient was poorly nourished; her weight on admission was 101 pounds. Insulinization was started on June 18, with 5 units before meals, and subsequently increased a few units at a time at intervals of a few days, until the patient was receiving 15 units t. i. d. on July 1. Blood sugars during the entire course were usually normal, sometimes somewhat lower. A glucose tolerance test was done before and immediately after the period of insulinization. The curve of the pre-insulin period was normal showing a maximum of 140 mg. at 1.5 hours and returning to 112 mg. at the end of two hours. The glucose curve obtained immediately after stopping the insulin showed a maximum of 192 mg. at the end of one hour, but had fallen to 82 mg. at the end of two hours; the urine was negative for sugar. Although readjustment to normal was prompt in this case, there was evidence of a transitory diminution of tolerance.

E. G., female, aged forty-four years, was admitted to the hospital June 7, 1932. Her complaints were weakness, loss of weight, and a poor appetite. A diagnosis of infectious arthritis and psychoneurosis was made. Insulinization was started on June 10 with 5 units before meals and was subsequently progressively increased to 18 units t. i. d. by June 25. Insulin was discontinued on June 30 with the last injection at noon. The blood sugar at

TABLE III  
I. N. HOSPITAL No. G582

DATE	BLOOD SUGAR		INSULIN BEFORE MEALS	BODY WEIGHT
	9 A.M.	2 P.M.		
	mg. per cent		units	pounds
5/ 3/32	120			120
5/ 4		140	5- 5- 7	120
5/ 5		92	7- 7- 7	120
5/ 6		122	7- 7- 7	120
5/ 7			7- 7- 7	119
5/ 8	140		7- 7- 7	121
5/ 9		104	7- 7- 7	121
5/10		110	7- 7- 7	120
5/11	85		7- 7- 7	120
5/12	182		7-10-10	120
5/13	95		10-10-10	120
5/14	98		10-10-10	120
5/15	122		10-10-10	120
5/16			10-10-10	120
5/17	114		10-10-12	121
5/18			12-12-12	121
5/19		138	12-12-12	122
5/20			12-12-12	122
5/21	147		12-12-12	122
5/22			12-15-15	125
5/23	78		15-15-15	125
5/24			15-15-15	124
5/25	253*	179		125
5/26	124	140		126
5/27	98			

\*Glycosuria 0.5 per cent.

TABLE IV  
E. H. HOSPITAL No. G4989

DATE	BLOOD SUGAR	INSULIN BEFORE MEALS	BODY WEIGHT	REMARKS
	mg. per cent	units	pounds	
6/18/32	109*, 1	0- 0- 5	101½	'Fasting; tolerance
6/19	118	5- 5- 7		
6/20	104	7-10-10		
6/21	95	10-10-10	102¾	
6/22	104	10-12-12		
6/23	59	12-12-12		
6/24	81	12-13-13		
6/25	84	13-14-14	101½	
6/26	66	14-14-14		
6/27	98	14-14-14		
6/28	110	14-14-14	100½	
6/29	79	14-14-14		
6/30	105	14-14-14		
7/ 1	96	14-15-15	101½	
7/ 2	82	15-15-15		
7/ 3	67	15-15-15		
7/ 4	110	15-15-15		
7/ 5	87	15-15-15	100	
7/ 6	100	15-15-15		
7/ 7	99	15-15-15		95 at 2 P.M.
7/ 8	94	15-15-15		
7/ 9	104*, 2			*Fasting; tolerance
7/10	100			
7/12			100	

\*Glucose tolerance: 50 gnl. glucose by mouth

Hours	0	0.5	1	1.5	2	
B. S. mg. per cent	109	112	135	140	112	Urine Negative
B. S. mg. per cent	104	157	192	109	82	Negative

Blood sugars were ordinarily taken at 9 A.M., two hours after breakfast.

end of two hours with no glycosuria, the curve obtained after insulinization showed considerably greater diminution of tolerance. By the second day after insulin was discontinued, the two-hour postabsorptive blood sugar level had returned to about its previous level. The patient's weight had increased from 108 pounds on admission to 112 pounds on June 28, and further increased after insulin was discontinued to 114 pounds by July 7. The patient's appetite was only slightly increased.

H. J., female, aged thirty-four years, was admitted to the hospital June 2, 1932. A diagnosis of pleurisy with effusion was made. She had lost weight and her appetite was poor. Insulin injections were started on June 4 with 5 units before meals, and the insulin was increased a few units at a time at intervals of several days to 25 units t. i. d. Blood sugar values were usually normal, but frequently higher. Insulin was discontinued on July 10 with the last injection at the evening meal. A glucose tolerance before the insulin injections were started was normal with a maximum of 159 mg. at 0.5 hour and 95 mg. at two hours. A second test was done on June 18 when the patient was receiving 10 units of insulin t. i. d.; the blood sugar reached a maximum of 231 mg. at one hour and was 100 mg. at the end of two hours. On July 11 when insulin was discontinued after the patient was receiving 25 units of insulin before each meal, the glucose tolerance curve was of the mild diabetic type reaching a maximum of 253 mg. at 1.5 hours, and was 204 mg. at two hours, and 176 mg. after 2.5 hours with a glycosuria of 0.8 per cent at one hour and 1.1 per cent at two hours. Another glucose tolerance curve five days after insulin had been discontinued showed a maximum of 208 mg. after one hour, but dropped to 99 mg. after two hours. The last tolerance done after eight days while showing a further improvement had not returned to the initial level; the maximum blood sugar was 173 mg. at one hour, and 109 mg. at the end of two hours. The patient's weight had increased from 104 pounds on admission to 113 pounds at the time insulin was discontinued; there was no subsequent gain in weight observed. Her appetite showed considerable improvement.

#### DISCUSSION

The observations that insulin may be given in progressively increasing amounts without causing hypoglycemia, and that a temporary hyperglycemia and glycosuria associated with a diminution of glucose tolerance is observed upon the sudden discontinuation of the injections suggest a certain endogenous readjustment of the organism to maintain a normal blood sugar level. The most obvious explanation is that there is a compensatory inhibition of the normal islet secretion during the course of insulinization, and a slow readjustment following discontinuation of the insulin until normal activity is attained. Such an interpretation is in accord with the evidence from this laboratory of a latent functional capability of the island cells in diabetes mellitus.<sup>8,9</sup> Similar observations indicating the suppression of the normal activity of a gland through the exogenous administration of its secretion have been reported with respect to the thyroid, parathyroid, and ovaries. However, a study of the respiratory quotients of insulinized nondiabetic patients has suggested another possible explanation. During and following periods of insulinization we have observed low respiratory quotients indicating an increased combustion of fat. Since Sweeney<sup>10</sup> has shown that high fat diets tend to reduce the glucose tolerance, this factor may possibly be operative in the experiments reported here.

The apparent increases in appetite and body weight have been of the greatest interest clinically. Most observers have reported substantial increases during as well as following a series of daily insulin injections. It is to be noted, however, that in many instances the total calorie intake was increased to as high



2 P.M. was 120 mg., but at 7 P.M. was 295 mg. with a glycosuria of 0.14 per cent. A glucose tolerance test the following morning gave a mild diabetic type of curve showing a maximum of 241 mg. at two hours and a glycosuria of 0.54 per cent at one hour and 0.22 per cent at two hours. Although the curve obtained before insulin was started showed a somewhat diminished tolerance with a maximum of 226 mg. at one hour, and 176 mg. at the

TABLE VI  
H. J. HOSPITAL No. G4997

DATE	BLOOD SUGAR	INSULIN BEFORE MEALS	BODY WEIGHT	REMARKS
	mg. per cent	units	pounds	
6/ 4/32	87*, <sup>1</sup>	0- 5- 5	104½	*Fasting; tolerance
6/ 5	79	5- 5- 5		
6/ 6	131	5- 5- 5		
6/ 7	105	5- 7- 7	106½	
6/ 8	86	7- 7- 7		
6/ 9	109	7- 7- 7		
6/10	91	7- 7- 7		
6/11	124	7- 8- 8	107½	
6/12	118	8- 9- 9		
6/13	105	9- 9- 9		
6/14	104	9-10-10		
6/15	85	10-10-10	109½	
6/16	94	10-10-10		
6/17	108	10-10-10	109½	
6/18	87*, <sup>2</sup>	0-10-10	110½	*Fasting; tolerance
6/19	77	10-10-12	109½	
6/20	109	12-15-15	110	
6/21	85	15-15-15	110	
6/22	159	15-18-18	110	
6/23	58	18-18-18	109½	
6/24	92	18-19-19	110½	
6/25	152	19-20-20	111½	
6/26	33	20-20-20		
6/27	94	20-20-20		
6/28	124	20-20-20	110½	
6/29	165	20-20-20		
6/30	114	20-20-20		
7/ 1	138	20-21-21	112½	
7/ 2	157	21-22-22		
7/ 3	155	22-23-23	116½	
7/ 4	110	23-23-23		
7/ 5	73	23-23-23	114½	
7/ 6	107	23-23-23		
7/ 7	159	23-24-24		
7/ 8	155	24-25-25		
7/ 9	90	25-25-25	113½	
7/10	82	25-25-25		
7/11	87*, <sup>3</sup>			*Fasting; tolerance
	140 (1 P.M.)			
	147 (2 P.M.)			
7/12	122		113	
7/13	155			
7/15	98			
7/16	114*, <sup>4</sup>		113½	*Fasting; tolerance
7/18	104			
7/19	91*, <sup>5</sup>			*Fasting; tolerance

\*Glucose tolerance: 50 gm. glucose by mouth

Hours	0	0.5	1	1.5	2	2.5	
*B. S. mg. per cent	87	159	126	124	95		Urine
*B. S. mg. per cent	87	182	231	179	100		Negative
*B. S. mg. per cent	87	208	231	253	204	176	Negative
							1 hr. 0.8%
							2 hr. 1.1%
*B. S. mg. per cent	114	204	208	189	99		Negative
*B. S. mg. per cent	91	186	173	147	109		Negative

Blood sugars ordinarily taken at 9 A.M., two hours after breakfast.

## STUDIES ON INDOLURIA\*

J. C. FORBES, M.A., PH.D., AND R. C. NEALE, RICHMOND, VA.

THE present investigation was begun in an attempt to determine whether there is any evidence of impairment in the processes of detoxification in the body in chronic arthritis. At the beginning of the investigation, we made quantitative estimations of the amount of indican excreted by patients with chronic arthritis, routine hospital cases excluding arthritic patients, and normal subjects. The amount of indican excreted by 43 normal subjects, as determined by the Sharlit<sup>1</sup> method, averaged 78 mg. per day. Usually determinations were made on twenty-four-hour specimens for three consecutive days. There was considerable variation in the amount found in different persons, the indican ranging from 30 to 165 mg. per day. The average for 157 hospital cases with various pathologic conditions other than chronic arthritis was 67 mg. per day, the amounts ranging from 5 to 260 mg. In the cases of arthritis there was a tendency for a low indican output, the average for 96 cases of arthritis being 44 mg. per day, the amounts ranging from 7 to 358 mg. Although the average indican output seems to be lower in chronic arthritis than in other pathologic conditions, no definite relationship was found to exist between it and the clinical condition of the patient. Hypertrophic cases were more consistently low than rheumatoid cases. The high figures were all found in the latter condition.

Owing to the inconsistency of our results with indican determinations, we turned our attention to the examination of urine for indole, believing that if there were impairment in the processes of liver detoxification, indole might appear as such in the urine. As the results show, indole or a precursor of indole which is converted into indole in an acid solution was found in the urine of most cases of chronic arthritis.

The presence of indole in the urine distillate of normal individuals was reported by Jaffé.<sup>2</sup> However, the amount was very small since one or two liters of urine were necessary for each determination. Similarly, we have found that when urine is distilled directly to a very low volume, as done by Jaffé, indole sometimes appears in the distillates from normal urines. It has also been our experience that the addition of strong mineral acids to the urine before distillation often leads to considerable indole formation. Although small amounts of indole, or of some substance which liberates indole on steam distillation from a solution made acid with an organic acid, are occasionally found in normal urine, they are not present in significant amounts. This is evident from the fact that out of over one hundred determinations on normal individuals, we found urinary indole in only one case and then on only one occasion.

\*From the Department of Biochemistry, Medical College of Virginia.  
Received for publication, December 15, 1934.

as 4,000 calories, so that it is to be questioned whether or not the increased caloric intake in itself would not have produced the same results. An increased gastric motility associated with an increased secretion of gastric juice following insulin injections<sup>11, 12, 13, 14</sup> has been reported by a number of investigators; this may in part explain the increased appetite frequently reported, especially in cachectic states. It would seem advisable that for purposes of stimulating the appetite, the insulin should be given an hour or more before meals. Whether exogenous insulin plays a definite rôle metabolically in producing an increase in body weight remains an open question.

#### CONCLUSIONS

Insulin may be given in progressively increasing amounts to nondiabetic subjects without producing hypoglycemia.

There is a progressive diminution of glucose tolerance accompanying the increase in the amount of insulin given.

Upon suddenly discontinuing the course of insulinization, there is a resulting transitory hyperglycemia and glycosuria lasting one to three days or even longer.

The data indicate that the insulin given may cause a compensatory inhibition of the normal islet secretion.

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chloroform extract up to the 12.5 c.c. mark. The solution then turned a greenish blue color. This was compared colorimetrically with a standard indole solution treated in the same way as the final distillate. A stock solution of indole was made by dissolving 20 mg. of indole C.P. in 100 c.c. of water. This solution will keep for a week or more in the ice box. The dilute standard was prepared from this solution by diluting 5 c.c. to 100 c.c. in a volumetric flask. For the determination 5 c.c. of the dilute standard was placed in a tube, further diluted to 50 c.c., and the color reagents added. The recovery of indole is not totally quantitative owing to incomplete distillation of indole, but the results obtained by the above method should be comparable. Furthermore, we feel that the important factor is the presence of appreciable quantities, and not the absolute daily output, of indole in the urine.

The results obtained from the various urines studied are given in Tables I and II. Values given as traces are less than 0.05 mg. per liter of urine. Very few of those cases giving a positive result showed as low as 0.3 mg. per liter. The majority of them were between 0.5 and 1.5 mg. per liter of urine. It is likely that traces of indole are not of clinical significance.

In Table I is shown the occurrence of indoluria in a number of different diseases. In Table II are shown the urinary indole output in typical cases of several diseases and the variations that accompanied clinical improvement.

TABLE I  
URINARY INDOLE IN VARIOUS CONDITIONS

NO. OF CASES	NEG.	TRACE*	POSITIVE	DIAGNOSIS
50	49	1	0	Normals
4	4	0	0	Bronchopneumonia
8	4	2	2	Lobar pneumonia
1	0	0	1	Abscess of lung
3	2	0	1	Peptic ulcer
2	0	0	2	Alcoholic intoxication
2	0	0	2	Acute yellow atrophy of the liver
13	2	0	11	Congestive heart failure
8	0	0	8	Pellagra
2	0	0	2	Cirrhosis of the liver
1	0	0	1	Exfoliating dermatitis
20	1	1	18	Pulmonary tuberculosis
1	0	0	1	Tuberculous peritonitis
12	4	8	0	Upper respiratory infection
8	0	0	8	Diabetes with gangrene
11	5	1	5	Diabetes without gangrene
14	2	0	12	Toxemia of pregnancy
2	0	0	2	Rheumatic fever
5	3	0	2	Gonococcal arthritis
3	0	0	3	Infectious arthritis
24	0	2	22	Rheumatoid arthritis
13	2	1	10	Hypertrophic arthritis
2	0	0	2	Mixed arthritis
34	32	2	0	Various pathologic conditions, such as acute lymphatic leucemia, psychoneurosis, syphilis, pleurisy, typhoid fever, carcinoma of the stomach, arsenical dermatitis, common cold, malnutrition, and Banti's disease.

\*A urine was considered to contain only a trace of indole if it showed less than 0.05 mg. of indole per liter of urine.

Becher,<sup>3</sup> upon examination of the urine, found free indole in cases of severe liver insufficiency. Cantelli<sup>4</sup> found indole in the urine of patients with destructive pulmonary lesions, mainly tuberculosis. He attributes indoluria in such cases to putrefaction in the body. Schonr and Rosengarten<sup>5</sup> also found that many cases of pulmonary tuberculosis gave a positive result to the test for indole in the urine. In their cases, the amount of indole present was somewhat proportional to the activity of the infection. Those patients with a large amount of indole in the urine usually had an unfavorable later clinical course, while those patients showing a negative or slightly positive test for indole progressed favorably.

Vaughan<sup>6</sup> tried to determine if the power of the liver to detoxify indole could be used as a liver function test. He based his work on the theory that if the liver were unable to detoxify indole because of intrinsic disease, free indole would appear in the urine. Therefore, he tested the urines of patients with various pathologic lesions of the liver, such as acute arsenical hepatitis, acute catarrhal jaundice, subacute yellow atrophy, obstructive jaundice, and jaundice of undetermined origin. He also tested urines from patients with marked intestinal stasis and putrefaction. In no case was he able to detect indole by the method employed, which was a modification of the test with Ehrlich's reagent. Bilirubin was precipitated from the urine with barium chloride, the solution filtered, and a petroleum ether extract of the filtrate treated with Ehrlich's reagent. The difference between Vaughan's results and ours (given below) may be attributed to the difference in the methods employed.

Since the presence of indole or of an indole precursor in the urine in cases of arthritis can hardly be considered as arising from a focus of putrefaction in the body or from a demonstrable liver insufficiency, it was decided to investigate the possible occurrence of indole in various other pathologic conditions in an attempt to explain its presence in arthritis. As our results show, indole was found to be present in most cases of arthritis, toxemia of pregnancy, pellagra, rheumatic fever, tuberculosis, and in many cases of diabetes mellitus.

*Experimental.—Method of Analysis:* One hundred cubic centimeters of fresh urine, usually the morning specimen, were placed in a 600 c.c. distilling flask, 20 drops of bromphenol blue added, and a 40 per cent aqueous solution of tartaric acid run in until there was an excess of 5 c.c. above the volume necessary to give a color change of the indicator. This usually required approximately 6 c.c. of the tartaric acid solution. The solution was steam distilled until 200 c.c. of the distillate was collected. A small flame was kept under the distilling flask during the distillation to maintain a relatively constant volume of liquid in the flask. The distillate was transferred to another distilling flask, the solution made alkaline to phenolphthalein, and redistilled over a free flame until 100 c.c. had distilled over. To 50 c.c. of this distillate, 1 c.c. of 2 per cent KOH was added, followed by 1 c.c. of approximately 2 per cent sodium B-naphthoquinone-4-sulphonate, and the solution mixed. After standing for ten to fifteen minutes, the solution was extracted with 3 c.c. of chloroform. The presence of indole is shown by a red color in the chloroform layer. The supernatant layer was syphoned off and alcohol containing 1 per cent KOH added to the

TABLE II—CONT'D

PATIENT	DIAGNOSIS	INDOLE	DATE	CONDITION
Miss Si.	Pellagra	--	3/ 3/34	Treatment begun, advanced case
		2.0	3/ 6/34	Very sick
		Trace	3/10/34	Very rapid improvement, skin lesions clearing
		0.1	3/17/34	Good
		0.2	3/18/34	Good
		Neg.	3/31/34	Symptom-free, excellent condition
Mrs. De.	Spondylitis	0.5	5/ 2/34	Moderately severe case
		0.3	5/ 5/34	Some improvement
		Trace	5/ 8/34	Continued improvement
Mr. Whi.	Congestive heart failure	2.4	7/ 8/34	In serious condition, much decompensation
		Trace	7/11/34	Given digitalis, with good response
		0.5	7/16/34	Very much better
		Neg.	7/18/34	Continued good In good condition
Mrs. St.	Toxemia of pregnancy	1.0	7/10/34	Serious condition
			7/11/34	Spontaneous delivery of normal child
		0.5	7/19/34	Improving rapidly
		0.4	7/21/34	Improving rapidly
		Trace	7/23/34	Continued to improve
		0.2	7/27/34	Good
I. T.	Toxemia of pregnancy	Neg.	7/29/34	Discharged in good condition
		Trace	7/13/34	Severe toxemia of pregnancy in fourth month
		2.8	7/15/34	Condition serious
		2.4	7/17/34	Unchanged
		0.5	7/18/34	Better
		0.7	7/18/34	Regaining strength
		0.9	7/19/34	Stronger
		--	7/30/34	Sent home to return for delivery
		0.8	11/ 7/34	Returned in labor, very sick
			11/ 8/34	Delivered
F. S.	Toxemia of pregnancy	Neg.	11/10/34	Very much better
		Neg.	11/11/34	Rapidly regaining strength
		--	11/25/34	Patient very ill
		--	11/26/34	Spontaneous delivery of normal child
		0.8	11/27/34	Uneventful and rapid recovery
		Neg.	11/29/34	Discharged on December 6
Mrs. H.	Toxemia of pregnancy	Trace	11/30/34	
		Neg.	12/ 1/34	
		Neg.	12/ 2/34	
		--	7/29/34	Spontaneous delivery of normal child
		1.1	7/30/34	Slow but gradual recovery after delivery.
		3.0	8/ 1/34	Discharged in good condition on August 9.
		0.4	8/ 2/34	
		1.2	8/ 4/34	
		3.5	8/ 5/34	
		2.0	8/ 6/34	
		1.1	8/ 8/34	

Discussion.—Indole may originate in the body from two sources: from bacterial decomposition of tryptophane in the intestinal tract and from putrefactive foci. Indole is normally detoxified in the liver by conversion into indoxyl and conjugation with monopotassium sulphate to form indican. Laroche and Desbordes<sup>7</sup> injected indole into the portal vein of dogs and determined at intervals the amounts of indole and of indoxyl present in the hepatic vein. Blood taken from the hepatic vein immediately after the injection of indole

TABLE II  
VARIATIONS IN URINARY INDOLE DURING TREATMENT

PATIENT	DIAGNOSIS	INDOLE	DATE	CONDITION
Mrs. Hu.	Acute lobar pneumonia	1.8	4/16/34	Serious
		4.8	4/19/34	Unimproved
		1.8	4/21/34	Better, temperature down
		1.6	4/25/34	Temperature normal
		0.6	5/ 1/34	In good condition, discharged
Mr. Co.	Lobar pneumonia	0.6	4/18/34	Temperature 106°, serious
		Neg.	4/19/34	Temperature normal, much improved
		Neg.	4/20/34	Continued to improve
Mrs. Bo.	Exfoliating dermatitis	3.2	6/ 5/34	Rash with swelling over entire body
		2.8	6/ 7/34	Slightly better
		0.9	6/16/34	Much better, skin clearing rapidly
		0.5	6/22/34	Continued improvement
		Neg.	7/ 5/34	Skin lesions absent
Mr. El.	Hypertrophic arthritis	0.7	6/20/34	Suffering considerably
		0.9	6/21/34	Unchanged
		0.5	6/22/34	Unchanged
		--	6/23/34	"Arthritis diet"
		Neg.	6/28/34	Improvement remarkable
		0.3	7/11/34	Reappearance of pains following laxity in dieting
		Trace	7/18/34	Much better
		Neg.	7/31/34	Some pain present following recent exposure to cold and damp
		0.5	8/ 7/34	Pains returned following temporary discontinuance of diet
Mr. Ne.	Rheumatoid arthritis	1.0	6/23/34	Pain very severe
		0.7	6/25/34	Unchanged
		1.0	6/26/34	Unchanged
			6/27/34	Pains more severe, placed on "arthritis diet"
		0.8	6/28/34	Feeling better
		0.7	6/30/34	Somewhat improved
		0.5	7/ 5/34	Some improvement
		0.1	7/ 6/34	No pain
		1.1	7/ 8/34	Reappearance of pain, cause unknown
		0.6	7/18/34	Pain only slight
		0.6	7/21/34	Pain slight
		Trace	7/27/34	In good condition
		Neg.	7/29/34	Entirely free from pain
		Neg.	8/24/34	Continued good
			10/ 1/34	Diet discontinued
		1.2	10/15/34	Pains returned, rather severe
Mrs. Ar.	Rheumatoid arthritis	1.1	10/16/34	Unchanged
		0.8	10/17/34	Unchanged, returned to diet
		0.3	11/ 6/34	Much better, but some pain present
		Neg.	12/ 6/34	In good condition, no pain
		0.7	6/ 7/34	Considerable pain
		0.5	6/ 8/34	Unchanged
			6/ 9/34	"Arthritis diet"
		Neg.	6/23/34	Free from symptoms
Mrs. Gr.	Hypertrophic arthritis	0.7	6/20/34	Suffering considerably
		0.9	6/21/34	Unchanged
		0.5	6/22/34	Unchanged
			6/23/34	Placed on "arthritis diet"
		Neg.	6/28/34	Improvement remarkable
		0.3	7/11/34	Some reappearance of pain, following laxity of diet
		Trace	7/18/34	
		Neg.	7/31/34	Some pain present following recent exposure to cold and damp
		0.5	8/ 7/34	Pain following temporary discontinuance of diet

origin, although the well-known toxic degeneration of liver cells in this condition is a more probable explanation. Another possible explanation for the indoluria of tuberculosis is that, due to the lowering of general resistance, the liver is unable to detoxify the indole absorbed from the intestinal tract so that some of it passes to the kidneys and appears in the urine. In diabetes with gangrene, the indoluria may be attributed to the putrefying and gangrenous area.

The explanation of indoluria in cases of congestive heart failure, severe liver damage, and toxemia of pregnancy is clear. Since the liver is the seat of indole detoxification, any impairment of its function by congestion or damage to the organ itself would be expected to allow indole to pass through unchanged.

The presence of indole in the urine in cases of arthritis, diabetes without gangrene, and pellagra is not so easily explained. It is not clear whether we are dealing with a greatly increased formation of indole in the intestinal tract or with a decreased power of the liver to detoxify the indole normally formed and absorbed or with a combination of both. The possibility of indole formation in some focus outside of the intestinal tract can hardly be considered a factor in these diseases. We are as yet in no position to prove or to disprove either of these possibilities.

It is of interest to note that when patients with chronic arthritis, pellagra, congestive heart failure, or toxemia of pregnancy have improved, there has been a reduction in the amount of indole excreted parallel, as a rule, with the clinical improvement. In several cases of toxemia of pregnancy, indoluria continued for several days after delivery even after the clinical symptoms of toxemia had apparently disappeared. We have, at present, no plausible explanation for this.

The mechanism by which the indoluria is eliminated in pellagra and arthritis is still unknown, but it is of interest to note that the ordinary pellagra diet, as well as the diet used in the treatment of the arthritic patients reported here, is high in both sulphur and vitamin B<sub>2</sub>. We are tempted to believe that, especially in arthritis, the high sulphur content of the diet was a factor in bringing about improvement. A detailed clinical report of our results on the treatment of arthritis with this diet will be published later.

#### SUMMARY

Urine from normal subjects and from patients with various pathologic conditions was examined for indole. Indole was found in significant amounts in many cases of chronic arthritis, pellagra, diabetes mellitus, tuberculosis, toxemia of pregnancy, congestive heart failure, and lobar pneumonia, but not in the urine of normal subjects. A short discussion dealing with the mechanism of indoluria is given.

We wish to extend our thanks to Eli Lilly and Company for a research grant which enabled R. C. N. to devote the major part of his time to research work.

The clinical diagnoses of the cases here reported were made by the clinical staff of the college hospitals. We take pleasure in acknowledging their cooperation in this matter, as well as for their many helpful suggestions during the investigation.



gave a positive test for indole but not for indoxyl. Ten minutes later indoxyl began to replace indole, large quantities of indoxyl being found in the hepatic vein even up to forty minutes after the injection of indole. Similar experiments were performed on excised liver, kidney, and lung tissues after perfusing them with Ringer's solution. The liver only was found capable of oxidizing the indole to indoxyl.

Sulphur is a major factor in the detoxification of indole. The conjugation of indoxyl with the sulphate radical forming the ethereal sulphate, indican, most likely takes place in the liver. Hele,<sup>8</sup> working with dogs, found that the ethereal sulphate was increased in the urine after administration of indole. He believes that the sulphur used by the body for detoxification is probably derived from the sulphate ions in the cell fluids, and that, as this is depleted, the sulphate ions are replaced by those formed from the oxidation products of metabolism. In the dog, this resulting sulphur deficiency can be replenished by the oral administration of sulphate, but in the pig, orally administered sulphates have no detoxifying action.<sup>9, 10</sup>

It is still unsettled whether sulphates as such are of value in the detoxification processes in man. Voegtlin, Johnson and Dyer<sup>11</sup> have shown that cystine, cysteine, and glutathione all protect the albino rat from minimal lethal doses of cyanide. Agnoli<sup>12</sup> found that colloidal sulphur exerted a definite antitoxic effect against minimal lethal doses of diphtheria and botulinus toxins. Waelseh<sup>13</sup> found that the narcosis produced by avertin could be avoided by the administration of glutathione or several other sulphur compounds. These investigations tend to show that certain sulphur compounds are important factors in the protection of the body against toxic compounds and in the elimination of these compounds from the body.

On examination of Tables I and II we find that indoluria may be present in patients suffering from the following pathologic conditions: lobar pneumonia, severe alcoholic intoxication, liver atrophy, congestive heart failure, pulmonary tuberculosis, diabetes mellitus with and without gangrene, pellagra, toxemia of pregnancy, and chronic arthritis. To explain the presence of indoluria in this list of diseases is not a simple matter. Several of these conditions are of questionable origin, and the presence of urinary indole in them may be the result of similar etiologic factors, as yet unknown. Theoretically, indoluria might result from at least three conditions, first, absorption of indole from the intestinal tract in such large amounts that the normal liver is unable to cope with it all; second, such impairment of the liver function that it is incapable of detoxifying even that amount which is absorbed normally from the intestinal tract; and third, indole arising from some area of the body other than the intestinal tract, and being excreted by the kidney without having passed through the liver.

Indole in the urine of tuberculous patients, as reported by Cantelli<sup>4</sup> and Schour and Rosengarten<sup>5</sup> and confirmed by us, may be due to a definite focus of putrefaction in the lung, as suggested by Schour and Rosengarten. Indole found in the urine of severe cases of lobar pneumonia might have a similar

laboratory service may fail to administer antitoxin or may release cases still harboring virulent *C. diphtheriae* to spread infection to others. Healthy carriers of virulent organisms may also not be recognized and continue to spread infection. Examinations in which diphtheroids are mistaken for *C. diphtheriae* may result in unnecessary quarantine with economic loss or loss of school attendance. A satisfactory settlement of this problem has been prevented in large measure because of reliance on such elusive and indefinite grounds as morphology. Nearly every experienced bacteriologist has his own rather definite opinion as to what is or is not *C. diphtheriae*, morphologically. Resort, therefore, to checking one person's observations against another's, while helpful, has too often been indecisive, for opinion is not fact. Testing the toxicity of a culture for guinea pigs has also its limitations in determining if a disputed organism is or is not *C. diphtheriae*, for nonvirulent strains are well recognized.

Fermentation reactions seem to offer a most practical means of differentiating *C. diphtheriae* from morphologically similar organisms.

#### PURPOSE OF STUDY

One purpose, therefore, of this study was to determine if possible the dependability of fermentation reactions in distinguishing *C. diphtheriae* from similar morphologic forms. In the study of fermentation tests, an attempt was made to determine if such reactions were constant; to try to explain discrepancies that were obtained by some investigators; to find out if fermentation reactions correlated with virulence tests; to distinguish between fermentation reactions of *C. diphtheriae* and diphtheroids; and to find out if sugar fermentations correlated with colonial and other distinctive characters of *C. diphtheriae* types.

Another phase of this study is concerned with the recognition of different varieties of *C. diphtheriae*.

#### CHARACTER AND SOURCE OF MATERIAL

All cultures used in this study were isolated from Löffler slant cultures inoculated from throat and nose swabs as routinely examined in the Bacteriological Laboratories of the Maryland State Department of Health. Since nose cultures were not routinely made for diagnosis in these laboratories until the past year, the majority of cultures studied have been from the throat. These cultures were secured from clinical cases of diphtheria, convalescents, contacts, and healthy carriers.

#### SOURCE OF CULTURES STUDIED

TABLE I

	NO. OF STRAINS	THROAT	NOSE	EAR
1. <i>C. diphtheriae</i>	111	107	4	0
2. Diphtheroids	50	47	2	1

#### METHOD OF ISOLATION OF PURE CULTURES

The isolation of pure cultures of *C. diphtheriae* is not always an easy matter. In stained preparations, contaminations may be so few in some instances

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## A STUDY OF CORYNEBACTERIUM DIPHTHERIAE AND RELATED ORGANISMS IN MARYLAND\*

ONA R. WHITLEY, M.S., BALTIMORE, MD.

### INTRODUCTION

THERE is considerable disagreement among bacteriologists as to the taxonomy of the species *C. diphtheriae*. Any attempt to classify this species solely on the basis of morphology is obviously impractical. No one can describe or picture the numerous variations that *C. diphtheriae* exhibits when grown on media of only slightly varying composition and in association with other organisms. While the organisms can, in many instances, be readily recognized solely by their morphology and staining characters on certain media, there are instances in the examination of Löffler slant cultures from the throat or the nose where it is impossible for the most skillful observer to be sure whether certain bacteria are *C. diphtheriae*, diphtheroids or neither. There can be no doubt that a certain percentage of cultures examined routinely in bacteriologic laboratories have been reported negative when *C. diphtheriae* has been present or reported positive when only diphtheroids were actually found. The question is how can one distinguish between *C. diphtheriae* and diphtheroids? Failure to diagnose *C. diphtheriae* may be serious, for a physician depending on

\*From the Maryland State Department of Health, Bureau of Bacteriology, and the Department of Bacteriology of the Johns Hopkins School of Hygiene and Public Health.  
Received for publication, November 30, 1934.

This paper is based upon and contains data from a thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Hygiene. The Johns Hopkins University, 1934.

trols. With the majority of strains of *C. diphtheriae*, definite though not always complete reactions were observed in twenty-four hours. Maximum reactions were reached within from forty-eight to seventy-two hours. The amount of acid produced by some strains from dextrin was considerably less than that formed from dextrose.

That *C. diphtheriae* ferments dextrose and fails to affect sucrose is recognized by most investigators. However, L. Martin (1898), Graham-Smith (1908), Moshage and Kolmer (1916), Cary (1917), Durand (1921), Fitzgerald and Doyle (1923), claim to have isolated *C. diphtheriae* strains which fermented sucrose. On the other hand, A. Knapp (1904), Zinsser (1907), Rothe (1907), Arkwright (1908), Morse (1912), Neisser and Gins (1913), Hine (1913), Guthrie, Gelien and Moss (1920), Jordan, Smith and Kingsbury (1922), Barrett (1923), Engering (1923), Christianson (1923), O'Kell and Baxter (1924), have failed to observe sucrose fermentation by *C. diphtheriae*.

In the present study, constant fermentation reactions were produced by all strains of *C. diphtheriae*. Dextrose, dextrin, and maltose were fermented by all the one hundred and eleven strains. Starch and glycogen were fermented by seven strains in addition to the above-mentioned sugars. Sucrose was not fermented by any of the *C. diphtheriae* strains. Dextrin was found to be weakly fermented by some strains. In some instances, it has been found to produce acid in twenty-four hours and then revert to an alkaline reaction. Both virulent and avirulent strains of *C. diphtheriae* fermented dextrin (see Table II). No difference was found in the fermentation reactions of virulent and avirulent strains of *C. diphtheriae*.

#### CULTURAL CHARACTERISTICS OF VARIOUS TYPES OF *C. DIPHTHERIAE* AND THE DIPHTHEROIDS

*Colony Morphology of Types of C. Diphtheriae and Diptheroids on Tellurite Agar Plates.*—Three types of *C. diphtheriae* colonies were observed on tellurite serum agar plates. These corresponded to the gravis, mitis and intermediate types described by Anderson, Happold, McLeod and Thomson (1931).

*Gravis colonies:* Colonies corresponding to the gravis type appear after forty-eight to seventy-two hours of incubation at 37° C. as dull, grayish black (nonglistening). They measured from 3 to 3.5 mm. when well isolated. The colonies were irregular in outline and had a tendency toward flatness and spreading. Radial lines were usually noticeable after forty-eight hours incubation and became more pronounced later. These features are responsible for the rough appearance of the gravis type of colony. *Mitis colonies:* Colonies corresponding to the mitis type were about the same size as the gravis but differed by being glistening black, round, and regular in outline and by having convex, smooth surfaces. *Intermediate colonies:* Colonies resembling the intermediate type were very small and dull gray with a raised black dot center at twenty-four hours. After forty-eight hours, the colonies became grayish black, rough and flat with a finely serrated edge. The intermediate colony usually measures about 0.5 mm. in twenty-four hours and from 1.00 to 1.25 mm. in forty-eight hours. The colonies are much smaller (about one-half the diameter)

that they may escape detection. Pleomorphism is so characteristic of *C. diphtheriae* that the contaminant though present may be taken for one of the individual cells of *C. diphtheriae* in the stained smear. On a few occasions, an apparently typical colony of *C. diphtheriae* has been found to contain a contaminating organism. The contaminants in such cases were staphylococcus, streptococcus or very rarely a diphtheroid. Hence, every culture studied has been carried through at least two subcultures before considering it pure. Insufficient care in purification and isolation has probably been responsible for some of the disagreements that different investigators have obtained in their studies on fermentation reactions.

In the isolation of cultures, infusion broth was inoculated with a loopful of material from Löffler slant containing the mixed culture and incubated at 37° C. for eighteen to twenty-four hours. A potassium tellurite plate was then streaked with a small loopful taken from the surface of the broth in such a manner as to secure *widely separated* colonies. Löffler medium was not used for plating directly from mixed cultures, as it was found subject to liquefaction and spreading by certain contaminating organisms, and too, colonies of *C. diphtheriae* were not so easily distinguished as on the tellurite medium. Several characteristic colonies from the forty-eight- to seventy-two-hour tellurite plates were picked to Löffler slants in order to secure different types if these were present and to avoid, if possible, the chance of selecting avirulent strains. As final proof that cultures were pure, tellurite plates were streaked from each individual Löffler slant culture. Portions of the same colony on a tellurite agar plate were transferred respectively to another Löffler slant to make ready for inoculation into fermentation tubes and to an agar slant for reserve. The agar slant culture was an additional check on purity since contaminants, if present, could be more easily detected in the growth on an agar slant which is thin, than on a Löffler slant which gives a more profuse growth.

#### FERMENTATION REACTIONS OF *C. DIPHTHERIAE*

The fermentation reactions of *C. diphtheriae* have been a controversial subject since the publication of Hine's statement in 1913 that he "had yet to find an organism of the diphtheria group giving acid with glucose and dextrin and not with saccharose which proves to be other than *B. diphtheriae*." It was found in this study that filtered sugars gave constant reactions, whereas heated sugars were unreliable in many instances. The following filtered sugars were used for the reactions: dextrose, dextrin, maltose, sucrose, starch, and glycogen. The reactions in Hiss serum water were found so inconstant and hence unreliable, in the first part of this study, that its use was discontinued and extract broth was substituted. After substituting extract broth containing 1 per cent of carbohydrate and indicator, no inconstant reactions resulted.

Transfers from twenty-four-hour Löffler slants were made into tubes containing the individual carbohydrates and incubated at 37° C. Readings were taken on the first four days and again on the seventh and tenth days. In some instances, readings were noted on the fourteenth, twenty-first, and twenty-eighth days. Uninoculated tubes were always incubated along with the tests as con-

On various batches of Löffler medium prepared under apparently the same conditions, however, variations in morphology of the same cultures have been enormous. In some instances, the change in morphology from batch to batch of medium has been so great as to make the identification of the cultures doubtful if not impossible if fermentations and other reactions had not been made. These variations have been found due to the method of preparation of the medium, the amount of moisture, the pH of the medium, the length of the incubation period, the portion of the slant from which it was taken, influence of other bacteria present and other possible factors. Some strains were found to have quite long forms on one batch of medium, other strains were of medium length, while some were very short. On some batches of Löffler medium, the very short forms would have been indistinguishable from diplococci if there had not been present a few pleomorphic forms. The long, medium, and short forms differed to some extent in their capacity to retain the methylene blue stain. Mitis strains took the stain rather deeply and uniformly with prominent metachromatic granules while other strains (gravis and intermediate) took the stain comparatively lightly and the metachromatic granules were relatively few or absent. Many variations of *C. diphtheriae*, such as dumb-bell, club, etc., may be seen in cultures at times. Usually the most typical morphology (pleomorphism) is obtained on Löffler medium which supports only scanty growth. While typical morphology is desirable for identification it is obvious that a medium supporting only scanty growth of *C. diphtheriae* is not entirely satisfactory for often good growth of other mouth or nose bacteria occurs so that it is almost certain in such instances that *C. diphtheriae* will be overgrown. Some pleomorphism must, therefore, be sacrificed in order to obtain reasonably satisfactory growth. With cultures of diphtheroids one form usually predominates. Rarely is this true of *C. diphtheriae*, for some variations in form and length are seen in any culture. It was not possible to group the strains according to Wesbrooke's classification.

*Morphology of C. Diphtheriae on Infusion Agar.*—On infusion agar, the morphology of *C. diphtheriae* cultures in general varied somewhat from that on Löffler medium in that the organisms were shorter, heavier, showed fewer metachromatic granules, and often showed a greater tendency toward pleomorphism. The gravis and intermediate strains in contrast to the mitis showed a morphology that was strikingly different in that they were much shorter, produced few or no metachromatic granules, staining was more uniform and a lighter blue. In other words, the gravis and intermediate morphology more closely resembled that of a diphtheroid (*C. xerosis*) than it did the mitis type of *C. diphtheriae* with its metachromatic granules, pleomorphism, and light and dark staining areas.

*Morphology of C. Diphtheriae on Potassium Tellurite Agar.*—The morphology of *C. diphtheriae* on tellurite agar was similar to that on infusion agar, except there was a more dwarfed appearance in the gravis and intermediate types in that spindle and bottle shapes were predominant.

*Growth of C. Diphtheriae in Infusion Broth.*—In infusion broth, the gravis type produced a definite pellicle, granular in structure. The supernatant broth was clear with a granular deposit. This contrasted to the mitis type which

than the other two types. They resemble those produced by streptococci. No variation was noticed in the colony morphology of any of the three types subcultured frequently over a period of seven months.

Colonies of diphtheroids showed much variation on tellurite serum agar, also. The majority of strains produced a small, dead white colony in twenty-four hours, which in forty-eight hours increased in size, became gray, smooth and moist. Other strains produced thin, transparent colonies of a light grayish brown coloring which in forty-eight hours became somewhat larger and duller in color. A few strains produced small flat, colorless colonies in twenty-four hours which, in forty-eight hours, became dry and brownish black in color.

*Colony Morphology of C. Diphtheriae and Diphtheroids on Löffler Medium.*—The general morphology of the three types of *C. diphtheriae* was similar on Löffler medium but was less characteristic than on tellurite serum agar as no color changes occurred.

The diphtheroid colonies like *C. diphtheriae* colonies were not so easily differentiated on Löffler medium as on the tellurite medium except when pigmented.

*Cellular Morphology of C. Diphtheriae and Diphtheroids on Löffler Medium.*—In this study, smears stained with methylene blue were made from cultures of *C. diphtheriae* grown on Löffler slants which had been incubated for eighteen to twenty-four hours. The cellular morphology of a single strain varied with the different batches of Löffler medium. It was found that the morphology varied all the way from "typical" *C. diphtheriae* to that of forms unrecognizable as *C. diphtheriae*. My experience has convinced me that a great deal of error has resulted in laboratories doing routine diphtheria work due to dependence for identification on cellular morphology of cultures grown on Löffler medium, the reasons for which will be pointed out later. The emphasis of textbook writers, teachers, and practical workers alike has been so much on cellular morphology that most laboratory workers have accepted an opinion based on cellular morphology and staining as beyond question. By checking identification of this sort against that based on fermentations, virulence, etc., errors of over 65 per cent have been encountered. There is great need for appreciation of the fact that the routine identification of *C. diphtheriae* is a difficult matter. It calls for painstaking study based not only on cellular morphology but also supplemented by study of cultural reactions. *Gravis type*: the gravis type did not produce the predominant, club-shaped (so-called typical *C. diphtheriae*) forms usually characteristic of the mitis type. This type usually took the methylene blue stain less heavily than the mitis type, and as a result the general impression was that of a lighter blue. Few or no metachromatic granules were produced by the gravis type. *Mitis type*: On the other hand, many metachromatic granules were usually found in the mitis type. As a rule, the mitis strains gave the "typical" *C. diphtheriae* morphology in that there was much pleomorphism, many metachromatic granules, characteristic arrangement, and much variation in length. *Intermediate type*: The morphology of the intermediate type was similar to that of the gravis type.

It is a well-known fact that the morphology of many strains of corynebacteria varies with the age of growth and the medium on which it is grown.

usual significance. As has been stated above, diphtheroids in pure culture can be differentiated from *C. diphtheriae* on morphologic grounds in the majority of instances as there are sufficient pleomorphism, characteristic arrangement and staining properties in *C. diphtheriae* to distinguish it from the uniformity, parallelism, and comparative evenness of staining of diphtheroids. On the other hand, in mixed cultures *C. diphtheriae* and diphtheroids seem to be more subject to variations in morphology than is seen in pure culture, and the personal element enters largely into the diagnosis of such cultures. It is in such cases as these where doubt exists as to the identity of certain forms that isolation of pure culture should be made and the fermentation reactions determined.

*Virulence of C. Diphtheriae and Diphtheroids.*—In reviewing the literature on the subject of virulence, it is apparent that there are many difficulties to be encountered before a definite opinion can be made as to the pathogenicity of a member of the corynebacterium group. Some investigators state that *C. diphtheriae* can be recognized by staining characteristics and morphology; others use sugar fermentations as a means of differentiation; but all agree that the final criterion of virulence is animal inoculation. Some investigators have considered all organisms resembling *C. diphtheriae* isolated from cases of diphtheria as of etiologic significance. It is possible that a culture may contain avirulent as well as virulent organisms, or it may contain only the avirulent organisms. In order to avoid the danger of selecting an avirulent strain of *C. diphtheriae*, several colonies were picked from the plate first inoculated.

Virulence was tested intradermally by injecting from 0.10 c.c. to 0.15 c.c. of a suspension of a twenty-four-hour growth from a Löffler slant in 5 c.c. of normal saline solution. Guinea pigs weighing between 300 gm. and 450 gm. were used for the tests. The control guinea pig received from 250 to 500 units of diphtheria antitoxin twenty-four hours previous to the time control and test guinea pigs were inoculated with the cultures. Four to six strains of the mitis type of *C. diphtheriae* were usually used on a guinea pig. The gravis and intermediate strains were tested singly. The range of virulence seemed to be greater in the mitis strains while the gravis were more uniform. Some mitis strains gave just as marked reactions on guinea pigs as the gravis and intermediate types, but they did not appear so toxic. Typical reactions with the mitis strains occurred when reddish halos with edema appeared around the point of injection and gradually faded into the natural color of the skin for about half an inch from the centers of injection. These color reactions appeared in twenty-four hours and reached their height in forty-eight to seventy-two hours, while controls showed no reaction. Autopsy findings for gravis strains revealed primarily congested and very hemorrhagic suprarenals with considerable serous fluid in the pleural and pericardial cavities. Guinea pigs inoculated with the intermediate strains showed the same reactions as for the gravis strains.

With regard to the virulence of the three types for guinea pigs, the results are in accord with those of Parish, Whitley and O'Brien (1932), in that some mitis strains are as pathogenic as the gravis strains (i.e., they kill just as quickly). However, on the whole it seems that the gravis strains appear



showed little or no pellicle, a moderately granular sediment, and a uniform turbidity in twenty-four hours. The intermediate type likewise showed little or no visible pellicle, had a clear supernatant broth, and had a finely granular sediment. After forty-eight hours' incubation, these observations were decidedly pronounced. The presence or absence of a pellicle can best be observed by holding the tubes up to the light and viewing them from below, diagonally.

*Growth of C. Diphtheriae and Diphtheroids on Infusion Agar Slants.*—Infusion agar was used as another medium on which to study the growth of the different strains of *C. diphtheriae*. A dull (nonglistening) heavy, rough growth was produced by the gravis type in contrast to the glistening, moderate, smooth growth of the mitis form. A sparse, nonglistening, rough growth similar to the growth of a streptococcus was formed by the intermediate type.

Though the growth of diphtheroids is usually indistinguishable from that of *C. diphtheriae* on infusion agar, at times the character of growth of certain diphtheroids has been very helpful in distinguishing them from *C. diphtheriae*. A thick growth whether moist, wrinkled, pigmented, or sticky is very unlikely to be a culture of *C. diphtheriae*.

*Hemolysis of C. Diphtheriae Cultures.*—Hemolysis of cultures of *C. diphtheriae* was determined by plates made with sheep blood. Very slight hemolysis was manifested by the gravis strains in twenty-four hours as contrasted with the mitis which produced a clear zone. However, on further incubation definite hemolysis was produced by the gravis type. This is in contrast to the findings of Anderson, McLeod, Haggold, and Thomson (1931). There was no evidence of hemolysis in the small number of intermediate strains studied. This lack of hemolysis has been suggested by Christison (1933) as a means of differentiation of the intermediate type. Hemolysis was produced by all of the mitis strains tested.

*Fermentation Reactions of C. diphtheriae and Diphtheroids.*—All fermentation reactions of the gravis, intermediate, and mitis types remained constant. With the gravis strains there was always strong acid production in dextrin, starch, and glycogen in twenty-four hours at 37° C. This appears to be a very constant and stable character and always correlated, in the strains studied, with the rough colony structure. The intermediate strains (few in number) gave the same sugar reactions as the mitis types in that they fermented dextrose, dextrin, and maltose. Sucrose was never fermented by any of the *C. diphtheriae* strains.

There is apparently no correlation between the cellular morphology of diphtheroids and certain fermentation reactions. The differential sugars that are of value are maltose, sucrose, and to a slight extent dextrose, which merely separates the nonfermenting group. Of the 50 diphtheroid strains, 8 fermented dextrose, maltose, and sucrose; 3 fermented dextrose and maltose; 3 fermented dextrose and sucrose; 13 fermented dextrose only; and 23 were non-fermenters (see Table III).

Dextrin was not fermented by any strains of diphtheroids so that the value of dextrin in differentiating the diphtheroids and *C. diphtheriae* has un-

mixed culture platings as well as in pure culture. In no instance was there a mixture of types on the plates from which the pure cultures were fished.

#### SUMMARY

1. In this study of *C. diphtheriae* and related organisms, forms similar to those described by Anderson, Happold, McLeod and Thomson (1931) have been found in Maryland. While only a few *gravis* (7) and intermediate (3) strains were isolated, the cultural reactions were as follows:

a. *C. diphtheriae gravis* grows with a granular deposit and pellicle in broth; ferments dextrose, dextrin, maltose, starch, and glycogen; forms large lusterless and flattened colony with radial lines; produces hemolysis.

b. *C. diphtheriae mitis* produces uniform turbidity in broth; ferments dextrose, dextrin, and maltose, and it fails to ferment starch and glycogen; forms entire, smooth, convex, glistening colony; produces hemolysis.

c. *C. diphtheriae intermediate* produces a fine granular sediment but little or no visible pellicle in broth; the supernatant is clear; ferments dextrose, dextrin, and maltose but does not ferment starch and glycogen; forms a small, rough, nonglistening, flat colony; produces no hemolysis.

2. The (3) forms of *C. diphtheriae* have remained constant even though transferred at intervals during a period of seven months.

3. Potassium tellurite plate medium was found of great value in differentiation of types. This medium was also found suitable for the growth of delicate as well as hardy forms of *C. diphtheriae*.

4. There was no evidence of mixtures of types of *C. diphtheriae* when original (mixed) cultures were plated out on the tellurite medium.

5. Fermentation reactions were constant for all *C. diphtheriae* strains. Dextrin was found to be weakly fermented by some *mitis* strains.

6. Fermentation reactions did not offer any differentiation between virulent and avirulent strains of *C. diphtheriae*.

7. Morphology does not distinguish between virulent and avirulent strains of *C. diphtheriae*.

8. It is difficult, if not impossible, to distinguish between *C. diphtheriae* and some diphtheroids in mixed cultures on morphology alone. The distinction is much less difficult in pure cultures.

9. Dextrin was found of value as a differential sugar for *C. diphtheriae* and diphtheroids.

10. No constant relationship was found to exist between morphology, fermentation reactions, and source of diphtheroids.

11. None of the diphtheroids tested showed any virulence.

12. Diphtheroids can frequently be distinguished from *C. diphtheriae* in mixed cultures on Löffler slants. Where doubt exists, the identity can nearly always be established after growth on a tellurite serum agar plate by colonial and cellular morphology. Rarely is it necessary to inoculate sugar broth tubes or make virulence tests to identify cultures correctly.

much more toxigenic or invasive, since all test guinea pigs died in forty-eight to seventy-two hours. The intermediate strains produced somewhat smaller amounts of edema, erythema, and necrosis on guinea pigs than the gravis strains, and they were not as toxic, as the guinea pigs did not die within seventy-two hours. In no case did any of the guinea pigs which were used as controls (i.e., injected with cultures and Park 8 antitoxin) die. Hence, it would appear that the antitoxin protects to the same extent for all types of *C. diphtheriae*. Parish, Whatley, O'Brien (1932) and Povitzky, Eisner and Jackson (1933) found the same results in testing the effectiveness of standard diphtheria antitoxin.

TABLE II  
CULTURES OF *C. DIPHTHERIAE*

NO.	AGAR SLANT	DEX- TROSE	DEX- TRIN	MAL- TOSE	SU- CROSE	STARCH	GLYCO- GEN	VIRU- LENCE
102 (Mitis)	Smooth, moderate white growth	A	A	A	-	-	-	93+ 9-
7 (Gravis)	Rough, heavy white growth	A	A	A	-	A	A	+
2 (Intermediate)	Rough, fine, white growth	A	A	A	-	-	-	+

TABLE III  
CULTURES OF DIPHTHEROIDS

NO.	DEXTROSE	DEXTRIN	MALTOSE	SUCROSE	STARCH	GLYCOGEN	VIRULENCE
23	-	-	-	-	-	-	-
13	+	-	-	-	-	-	-
3	+	-	+	-	-	-	-
3	+	-	-	+	-	-	-
8	+	-	+	+	-	-	-

That the morphology of *C. diphtheriae* has no known relation to virulence has been often demonstrated, and as has been stated above, cultural reactions also do not distinguish between virulent and avirulent *C. diphtheriae*. Hence it seems that virulence, so far, can only be determined by animal inoculation. In testing virulence of *C. diphtheriae* cultures, the intracutaneous method was found to be as reliable as the subcutaneous method. In the intradermal tests made, there were nine avirulent mitis strains. One of these was from a diagnostic case and the others were from healthy carriers.

Intracutaneous tests were made on all diphtheroid cultures. These tests were carried out in the same manner as for *C. diphtheriae* cultures. There was no indication of virulence in any of the cultures tested in this way.

*Correlation of C. Diphtheriae Types with Clinical Symptoms.*—Even though a comparatively small number of strains of gravis and intermediate types have been found in this study, there appears to be some correlation of morphologic colony types and clinical symptoms. All gravis and intermediate strains were isolated from fatal, very severe cases, or family contacts. These cultures showed very definite and characteristic rough, radial colonial morphology in

## MINOR HEMAGGLUTININS\*

### STUDY OF A SINGLE HUMAN BLOOD CONTAINING AUTOAGGLUTININ, HETEROAGGLUTININS, AND HEMOLYSINS, AND A ROULEAU- FORMING SUBSTANCE

WILLIAM P. BELK, M.D., PHILADELPHIA, PA.

**H**UMAN blood agglutinins (other than the familiar isoagglutinins) have been the subject of a number of interesting studies.<sup>1-11</sup> These include the heteroagglutinins (and hemolysins); subgroups of the isoagglutinins; the normal cold agglutinin of Landsteiner; the pathologic cold agglutinin, or panagglutinin, or autoagglutinin; and the rouleau-forming substance (or property). The distinguishing characteristics of the several substances are not entirely agreed upon. In particular a difference of opinion exists as to whether the pathologic cold agglutinin and the rouleau-forming substance are the same. Landsteiner's normal cold agglutinins and the pathologic cold agglutinins do not clearly differ except that the latter are often of high titer, and the former have some of the characteristics of the subgroup isoagglutinins. The presence of four of these substances in a single human blood offers an unusual opportunity to compare their characteristics. The subgroup isoagglutinins, however, are not here considered.

The subject was a white male of twenty years, otherwise healthy, who suffered a typical attack of infectious mononucleosis of seventeen days' duration. Recovery was uneventful. He had no history of allergy. His blood was Type II. Three samples of blood were obtained during the first month of convalescence for the present study. As the reactions were practically the same in all three, they are reported as one study. The heterophile sheep antibody titration according to the method of Paul and Bunnell<sup>12</sup> at the height of the illness was 1:32 for complete hemolysis and 1:256 for the agglutination.

*Technic.*—The subject's venous blood was allowed to clot at 37° C., and the serum removed before more than slight chilling could occur. This precaution was necessary to preserve the autoagglutinin in full strength in the serum, as this agglutinin adheres to the cells when chilled. The horse and sheep cells were from defibrinated blood and were several days old; the other cells were received at once into a 1.5 per cent solution of sodium citrate in normal saline. All cells were washed twice in warm (37° C.) saline and were used in a suspension of 1 per cent. Sterile precautions were used in preparing and keeping the materials, and all tests were done on the day of collection of the blood, or the day following.

\*From the Department of Clinical Biochemistry, Graduate School of Medicine, University of Pennsylvania.  
Received for publication, November 6, 1934.

NOTE: Further study of *C. diphtheriae* strains is being made, which will be reported later.

**Acknowledgments.**—I wish to express sincere appreciation to Dr. W. W. Ford, Professor of Bacteriology of the School of Hygiene and Public Health; to Dr. S. R. Damon, Associate Professor of Bacteriology and to Dr. Martin Probstner, Jr., Associate in Bacteriology of the School of Hygiene and Public Health of the Johns Hopkins University for their advice and criticisms in this study. Grateful appreciation is also expressed to Dr. C. A. Perry, Chief of the Bureau of Bacteriology of the Maryland State Department of Health, under whose supervision this work was done, for the many helpful suggestions and criticisms. Thanks are extended to the bacteriologists of the Central and Branch Laboratories of the Maryland State Department of Health for the cultures used in this work and for the information concerning the histories of the individuals from whom they were obtained.

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## HETEROAGGLUTININS AND HETEROHEMOLYSINS

*Protocol 3.*—The following titrations were done with Solution A from unheated serum, no complement added. Dilutions 1:4 to 1:256 were made (see Table II).

TABLE II  
HETEROAGGLUTININS AND HETEROHEMOLYSINS

	1 HR. 37° C.	1 HR. 10° C.	1 HR. 37° C.
	TITER	TITER	TITER
<i>Sheep cells:</i>			
Complete hemolysis	1:16	1:32	1:32
Partial hemolysis	1:32	1:64	1:64
Agglutination	1:64	1:256	1:256
<i>Horse cells:</i>			
Complete hemolysis	1:16	1:16	1:16
Partial hemolysis	1:32	1:128	1:128
Agglutination	1:256+	1:256+	1:256+
<i>Rabbit cells:</i>			
Complete hemolysis	1:8	1:8	1:8
Partial hemolysis	1:16	1:16	1:16
Agglutination	1:256	1:256+	1:256+
<i>Pig cells:</i>			
Complete hemolysis	1:16	1:16	1:16
Partial hemolysis	1:64	1:64	1:128
Agglutination		1:256	1:256

The same set-up was carried successively through the different temperatures. Saline controls of all cells were negative for agglutination and hemolysis.

Agglutinations were repeated with sheep and pig cells in Solution A from heated serum. The titer for both was 1:128, one tube lower. This was not considered a significant difference.

*Protocol 4.—Absorption:* One cubic centimeter portions of Solution A from heated serum were absorbed with about 0.5 c.e. amounts of cell mush from sheep, guinea pig, horse, and rabbit at 37° C. for one hour with several shakings. After centrifuging the supernatant, serums were pipetted. Each of the four serums was titrated with fresh cells of all four species, making 16 titrations in all, the dilutions being from 1:4 to 1:64. Guinea pig complement, 0.5 c.e. of 1:30 dilution, was added to each tube (see Table III).

The same set-ups were carried successively through the different temperatures. Saline controls of all cells were negative for hemolysis and agglutination. Titers are those for partial hemolysis, as complete hemolysis did not occur in every series.

The sheep cell agglutinins were not completely absorbed. The titers are a little lower than in unheated serum. This, however, was not considered a significant difference due to heating (see note Protocol 3). It is possible that less complement was added here than was contained in the original serum.

With heated serum collected on another day the same absorptions were carried out and the agglutinations allowed to proceed on slides. The specific absorption of the agglutinins was clearly demonstrated. When lecithin was added to each of the mixtures, no change was noted in the results.

Titration was done by the usual method of progressively doubling the dilutions of the serum and adding an equal amount of the cell suspension (0.5 c.c.). Agglutination was determined by placing a drop of the well-mixed suspensions under the microscope. The titers read one to two tubes higher than when read with the naked eye. The slide method of observing agglutination, the reversal of agglutination and the appearance of rouleaus, consisted of making light suspensions of cells in serum (about 1 per cent) on clean microslides ringed with a wax pencil. Slides placed in the 37° C. incubator were within a moist chamber to prevent drying. These and the slides in the refrigerator were taken out for observation one at a time so that the results could not be affected by a change in temperature to that of the room. Observations were made with the microscope, although the agglutinations were easily visible to the naked eye.

Lecithin has been shown by Lattes<sup>13</sup> to prevent rouleau formation. It was used here as a rather heavy suspension of a pure product in normal saline.

The serum and cells of the subject are designated as R serum and R cells. Type IV is that of the Moss classification.

#### ISOAGGLUTININ

*Protocol 1.*—Two 5 c.c. portions of R serum, one heated to 56° C. for thirty minutes, and one unheated, were absorbed with 1 c.c. of R cell mush (packed cells) at 5° C. for one hour. The mixtures were shaken several times. They were centrifuged in tubes surrounded by crushed ice and the serum pipetted off quickly. These are designated Solutions A. They failed to agglutinate R cells and Type IV cells after thirty minutes at 5° C., although moderate rouleau formation was noted. Removal of the autoagglutinin was considered complete (see Table I).

TABLE I  
TITERS WITH TYPE III CELLS

	37° C. 1 HR.	10° C. 1 HR.	37° C. 1 HR.
Sol. A heated	1:16	1:128	1:128
Sol. A unheated	1:32	1:64	1:64

The same set-up was carried successively through the different temperatures. Saline controls were negative.

*Protocol 2.*—Lecithin, R serum agglutinated Type III cells almost completely after thirty minutes at 37° C. on the slide. The addition of lecithin later had no effect in releasing the clumps. The agglutination was likewise unaffected when the lecithin was mixed with the serum and cells before incubation. The observations were repeated with the same results at a temperature of 10° C. It was observed, however, that when lecithin had been added, the clumps of cells were rounder, cleaner cut, and less ragged.

*Conclusions.*—The isoagglutination is of high titer. It is not apparently affected by a temperature of 56° C. for thirty minutes. It is apparently slightly stronger at low temperatures. It is not reversible. It is not affected by lecithin. This blood contains no autohemolysin.

*Conclusions.*—Four differently absorbable and specific heteroagglutinins and heterohemolysins were demonstrated. The agglutinins were not affected by a temperature of 56° C. for thirty minutes. The titers of both agglutinins and hemolysins were increased slightly with lowering of the temperature. There was no clear evidence of reversal of the agglutination reaction. The titer of the hemolysins was not increased by warming after chilling. The heteroagglutinations were not affected by the addition of lecithin. The titers of the hemolysins were possibly within the normal human range.

#### AUTOHEMAGGLUTINATION

*Protocol 5.*—The following agglutinations were done on all of the three samples of R's blood, a Type II blood. The serum was unheated and unabsorbed. The results were identical in each instance (see Table IV).

TABLE IV  
TEST FOR AUTOHEMAGGLUTINATION

MIXTURE	27-33° C. AGGLUTINATION	5-10° C. AGGLUTINATION
R serum + R cells	0*	+½
IV serum + IV cells	0	0
R serum + IV cells	0*	+4
IV serum + R cells	+4	+4†
Saline + R cells	0	0
Saline + IV cells	0	0

\*Slight rouleau formation.

†Indicating isoagglutination.

A portion of the serum heated to 56° C. for thirty minutes gave the same results with R cells and IV cells. In the chilled mixtures the agglutination reversed after a few minutes at room temperature, leaving definite rouleaus in two of the specimens. In the other specimen the reversal was slight or absent. This will be commented upon later.

*Protocol 6.*—*The effect of dilution on the autohemagglutinin:* R serum diluted 1:3 with saline agglutinated R cells + 1 and IV cells + 2. The actual titer of this serum with R cells was 1:4.

*Protocol 7.*—*Absorption of autohemagglutinin:* Equal parts of R serum and R cell mash were placed at 5° C. for two hours and shaken several times. The mixture was centrifuged in a tube surrounded with crushed ice, and the serum quickly pipetted. This is designated Solution A. The remaining cells were not washed. To them was added an amount of warm saline equal to that of the serum removed, and the tube placed at 37° C. for thirty minutes. The mixture was shaken several times. It was then centrifuged in a water jacket at 40° C. and the supernatant fluid pipetted. This is designated Solution B.

The following mixtures were made on slides and agglutination observed after thirty minutes at 8° C. (see Table V).

The agglutination quickly reversed at room temperature (26° C.). There was no evidence of rouleau formation.

*Protocol 8.*—*The effect of autohemagglutinin on heterologous cells:* Of R serum heated to 56° C. for thirty minutes 0.5 c.c. portions were absorbed with



TABLE III  
EFFECTS OF ABSORPTION

	1 HR. 37° C.	1 HR. 10° C.	1 HR. 37° C.
	TITER	TITER	TITER
<i>Sheep Absorbed Serum:</i>			
Sheep cells:			
Hemolysis	0	0	0
Agglutination	0	1:32	1:16
Pig cells:			
Hemolysis	0	1:8	1:8
Agglutination	0	1:8	0
Horse cells:			
Hemolysis	1:16	1:32	1:32
Agglutination	1:64	1:64	1:64
Rabbit cells:			
Hemolysis	1:16	1:16	1:16
Agglutination	1:64	1:64	1:64
<i>Pig Absorbed Serum:</i>			
Sheep cells:			
Hemolysis	1:32	1:64	1:64
Agglutination	--	1:32	1:32
Pig cells:			
Hemolysis	0	0	0
Agglutination	0	0	0
Horse cells:			
Hemolysis	1:32	1:64	1:64
Agglutination	1:64	1:64	1:64
Rabbit cells:			
Hemolysis	1:16	1:16	1:16
Agglutination	1:32	1:64	1:64
<i>Horse Absorbed Serum:</i>			
Sheep cells:			
Hemolysis	1:32	1:64	1:64
Agglutination	--	--	--
Pig cells:			
Hemolysis	1:4	1:8	1:8
Agglutination	1:4	0	0
Horse cells:			
Hemolysis	0	0	0
Agglutination	0	0	0
Rabbit cells:			
Hemolysis	1:8	1:16	1:16
Agglutination	1:64	1:64	1:64
<i>Rabbit Absorbed Serum:</i>			
Sheep cells:			
Hemolysis	1:32	1:64	1:64
Agglutination	--	1:64	1:64
Pig cells:			
Hemolysis	1:4	1:4	1:4
Agglutination	--	1:64	1:64
Horse cells:			
Hemolysis	1:32	1:64	1:64
Agglutination	1:64	1:64	1:64
Rabbit cells:			
Hemolysis	0	0	0
Agglutination	0	0	0

agglutinin was lost. This is mentioned to illustrate the great difficulty of working with this extremely thermolabile substance and the ease with which it may be overlooked in studies of serum.

*Protocol 10.—Rouleau-forming substance:* Rouleaus were observed in several tests of Solution A and in the mixtures of R serum with R and IV cells. The rouleau formation was never more than +2. In all these instances the addition of one or two 3 mm. platinum loopfuls of lecithin dispersed the rouleaus. This effect was clear-cut and complete, but did not occur until two or three minutes after the addition. The appearance of rouleaus was definitely more marked in chilled mixtures, and after such mixtures were warmed, and was usually absent in the same serum-cell combinations at room temperature and at 37° C. It is commonly stated that rouleaus are easily broken up by stirring, but this was not the case in these studies. The rouleau-forming substance was not found to be absorbable in any instance. Lecithin changed the shape of the erythrocytes by making them smaller and perfectly round in outline as viewed in one dimension.

#### COMMENT

Some observers consider autoagglutination an exaggeration of the rouleau-forming property. In the present instance the evidence is clearly to the effect that they are different, and that autohemagglutination is the result of a true agglutinin. The rouleau-forming property was not absorbable and was inhibited by lecithin, while the opposite was true of the autoagglutinin. In my experience clumping of red cells by the rouleau-forming property cannot always be distinguished from that of true agglutination by microscopic inspection, as advocated by Coea.<sup>14</sup> The action of lecithin appears to differentiate these two types of clumping quite clearly.

There is considerable resistance to the idea that an individual can develop an agglutinin against his own cells, in spite of numerous reports of human autohemagglutination. This agglutinin, however, is not specific in the sense that the isoagglutinins are, nor is it even species specific, but has the power of agglutinating the erythrocytes of many, possibly all, animals. An autoagglutinating property has been demonstrated in a large majority of the bloods of normal individuals by Landsteiner<sup>1</sup> and by Kettle.<sup>4</sup> Under certain conditions a powerful autoagglutinin appears, titers up to 1:250 having been reported.<sup>5, 6, 7</sup> It seems reasonable to suppose that these pathologic cold agglutinins are really Landsteiner's normal cold agglutinins increased in titer. The clinical states most often associated with these high titers are hemolytic jaundice, hemolytic anemia, some forms of cirrhosis of the liver, trypanosomiasis, repeated blood transfusions (in animals)<sup>3, 5, 15, 16, 17</sup> etc. In an experience of several hundred cross agglutinations requiring transfusions, and have seen sixteen posttransfusion reactions attributable to no other apparent cause. Most of these patients were sufferers from some hemolytic condition, chiefly from infections by hemolytic streptococci and staphylococci. Thus, in most of the diseases associated with autoagglutination, the destruction of red blood cells within the body seems to be a common factor.

TABLE V  
TEST FOR ABSORPTION OF AUTOHEMAGGLUTININ

	SOLUTION A AGGLUTINA- TION	SOLUTION B AGGLUTINA- TION
R cells	0	+4
IV cells	0	+4

approximately equal amounts of cell mush of guinea pig, rabbit, sheep and horse and Type III human cells at 37° C. for one hour. Mixtures were shaken several times. Mixtures were centrifuged at room temperature and serums pipetted. Mixtures were made on slides of each serum and fresh cells of the type used in the absorption (see Table VI).

TABLE VI  
THE EFFECT OF AUTOHEMAGGLUTININ ON HETEROLOGOUS CELLS

MIXTURE		37° C. AGGLUTINATION	5° C. AGGLUTINATION
Pig	absorbed serum + pig cells	0	+3
Rabbit	absorbed serum + rabbit cells	+2	+4
Sheep	absorbed serum + sheep cells	0	+4
Horse	absorbed serum + horse cells	0	+4
Type III	absorbed serum + Type III cells	0	+4

Saline controls of all cells were negative. The heteroagglutinin for rabbit cells was evidently not completely absorbed. The agglutination in all the other mixtures promptly reversed at room temperature (33° C.).

*Protocol 9.—The effect of lecithin on autohemagglutination is shown in Table VII.*

TABLE VII  
EFFECT OF LECITHIN ON AUTOHEMAGGLUTINATION

MIXTURE	AGGLUTINATION
R serum + R cells + a drop of lecithin	+3
R serum + IV cells + a drop of lecithin	+3
Saline + R cells + a drop of lecithin	0
Saline + IV cells + a drop of lecithin	0
On slides at 10° C. for 30 minutes.	

*Conclusions.*—An autohemagglutinin was demonstrated for the subject's own cells, for Type IV human cells, for cells of guinea pig, sheep, horse, and probably of rabbit. The agglutinin was not effective at room temperature (28° C.) but was strongly effective under 10° C. It was not altered by a temperature of 56° C. for thirty minutes. Its reaction was completely reversible with increase in temperature. It was absorbable. It was not affected by lecithin. It appeared not to be reversible in the presence of other agglutinating factors such as heteroagglutinin and the rouleau-forming substance.

Two attempts were made to isolate the autoagglutinin in pure solution by washing in ice cold saline the cells remaining from Solution A and centrifuging in cups packed with brine. In both instances the temperature of the mixtures rose to between 15° and 20° C. before the process could be completed, and the

# INFLUENZA VACCINE IN THE TREATMENT OF CHRONIC ENCEPHALITIS\*

RESULTS OF A STUDY AS TO A POSSIBLE RELATIONSHIP OF THE PFEIFFER BACILLUS  
AND EPIDEMIC ENCEPHALITIS

JOSEPH L. ABRAMSON, M.D., AND GEORGE VICTOR, M.D., BROOKLYN, N. Y.

THE question of a possible relationship between influenza and epidemic encephalitis has been a mooted point since the pandemic of the former in 1918 and the appearance of the latter at approximately the same time. It was in that year that von Economo in Vienna and Netter, Hall and others, in adjacent countries, called attention to a group of cases which we have since come to know as epidemic encephalitis. In the wake of these diseases there began to appear a train of symptoms which have been designated as chronic encephalitis, the most common form of which is Parkinsonism.

In view of the fact that many of the patients presenting the picture of Parkinsonism gave no history of an antecedent acute encephalitis but had had the "grip," the "Spanish influenza," the "flu," or an upper respiratory infection, some investigators suspected a causal relationship between influenza and epidemic encephalitis. Crookshank,<sup>1</sup> one of the strongest advocates of such a relationship, was of the opinion that "The synthetic view, then, is that encephalitis lethargica represents a nervous form of influenza. . . ." He brought forth numerous historical references to substantiate his opinion and referred to the work of Tarozzi who also held the same view. Volpino and Racehiusa<sup>2</sup> advanced strong experimental evidence in support of such a relationship. Jakseh-Wartenhorsch<sup>3</sup> reported seventy-three cases of chronic encephalitis which he designated as postinfluenzal encephalopathy. Felsani<sup>4</sup> and Reichelt<sup>5</sup> did likewise in their series of cases. Zibordi,<sup>6</sup> in three children suffering from epidemic encephalitis, obtained a positive reaction to intradermal injections of influenza vaccine and to no other. Crafts,<sup>7</sup> too, leans strongly toward a relationship between the two diseases.

However, such investigators as Stern,<sup>8</sup> von Economo,<sup>9</sup> Flexner,<sup>10</sup> Jordan,<sup>11</sup> Strümpell<sup>12</sup> and others are of the opinion that epidemic encephalitis is an entirely distinct clinical entity with no direct relationship to influenza. In its survey of epidemic encephalitis the first report<sup>13</sup> of the Matheson Commission concluded, "It is apparent that the weight of opinion is either against the identity of influenza and epidemic encephalitis, or a direct relationship between the two diseases."

Many authors, nevertheless, consider favorably the possibility of some indirect relationship. Hall<sup>14</sup> expressed himself as being of this opinion. Flexner

\*From the Neurological Service of the Jewish Hospital and the Jewish Sanitarium for Chronic Diseases, and the Department of Pathology of the Jewish Hospital.

This suggests that some product of the cell destruction acts as a stimulus (antigen) to the formation of the autohemagglutinin (panhemagglutinin).

The development of heterophile antibodies is usually attributed to the introduction into the animal body of the Forssman antigen. This idea is not controverted by the fact that the source of the antigen is not known (as in this case) since the antigen resides in many known, and probably in many unknown substances. Not all the facts of the present study, however, can be explained quite in this manner. To do so one would have to postulate the introduction of a Forssman type of antigen for horse, rabbit, and guinea pig erythrocytes as well as for those of the sheep, that is, an antigen with four different receptors, or else four different antigens simultaneously. The multiple production of heterophile antibodies from a single stimulus has been reported elsewhere. Deicher (quoted by Davidsolm<sup>15</sup>) found agglutinins for sheep, horse, rabbit, guinea pig, pig, and ox red cells in 90 of 102 human beings injected with horse (a few with sheep) serum. These agglutinins were specifically absorbable. Hanganutziu<sup>16</sup> found agglutinins for a series of animal red blood cells in about equal titer in 12 human beings injected with horse serum. These animals included sheep, horse, guinea pig, rabbit, veal, and pig. The suggestion that the heterophile sheep antibodies are Forssman stimulated, the others nonspecifically stimulated, hardly seems reasonable. A more plausible explanation, perhaps, is that of a powerful nonspecific stimulus to a general production of antibodies, the variety of these antibodies corresponding in a given case to such antigens as the cells have previously been "sensitized" to, or have the ability to produce naturally. Mino (quoted by Debenedetti) accounts for the appearance of panagglutinins on the basis of general stimulation. Kolmer<sup>20</sup> points to the fact of nonspecific stimulation of bacterial antibodies subsequent to an original specific stimulation, and suggests that the heterophile antigen may increase the production of natural sheep hemolysin nonspecifically. Bacterial agglutinins have been produced in rabbits by injection of heterophile antigen.<sup>9</sup> Thus the findings in the present study strongly suggest the existence of an unknown agent which stimulated the body nonspecifically to an excessive and apparently purposeless outpouring of antibodies. If this is found to be typical of all cases of infectious mononucleosis, then the disease is characterized, not so much by the presence of heterophile sheep antibodies, as by the appearance of many antibodies of various sorts.

#### CONCLUSIONS

A single human blood (from a convalescent case of infectious mononucleosis) was shown to contain, in addition to a high titer isoagglutinin, an autoagglutinin, four specific heteroagglutinins, four specific heterohemolysins, and a rouleau-forming property.

The characteristics of the several substances were demonstrated.

Some practical application of the findings to the problems of transfusion of blood have been discussed.

The study suggests the probability of the purposeless and excessive production of a number of antibodies simultaneously under certain conditions of non-specific stimulation.

legs, the posture became more erect, the face more mobile and the speech less monotonous. No febrile reactions were noted. Stewart concluded that 78 per cent of her patients with chronic encephalitis showed definite decrease in rigidity. The tremors either were not affected or were often increased.

The results obtained by Stewart and Evans seemed to indicate a definite relationship between the Pfeiffer bacillus and epidemic encephalitis and, as such, are of great importance especially as we assume that this bacillus is the cause of influenza. We accordingly felt that their results warranted further investigation.

We have under our supervision approximately 100 patients with chronic encephalitis. Seventy of these are ward patients who have been observed from one to five years and whose activities are regulated. These patients are in a hospital for chronic diseases, a fact which predicates that they could not be cared for at home or could not adequately care for themselves. Some have been bedridden for many years. Most of them, however, are up and about with varying degrees of incapacitation. In the course of their residence in the hospital all have received at one time or another the tincture of stramonium in the dosage recommended by Jacobson and Epplen,<sup>20</sup> dature stramonium gr. iv t. i. d. or more often, hyosine hydrobromide gr. 1/100 several times daily, atropine sulphate solution 0.5 per cent by mouth or by injection. Some of the patients have derived varying degrees of benefit from all or some of these drugs at one time or another. Because of the length of time they have been under our care and the control we have had over their medication, we have been able to establish a level for their physical state beyond which they did not seem able to go. Twenty-seven patients were seen in the Neurological Out-Patient Department of the Jewish Hospital. Most of them have been coming to the clinic for over three years at fairly regular intervals of two to three weeks. They, too, have received the various medications, except that none had been given any injections.

Nasopharyngeal cultures were made of all the patients. The medium used for cultivation was "chocolate agar" made by adding human red blood cells to heated nutrient agar of pH 7.4. The plates were incubated for twenty-four to forty-eight hours at 37° C. and the resulting growth examined with a hand lens for suspicious colonies. Subcultures of suspicious colonies were made on chocolate agar tubes and again on plain agar tubes. Organisms which showed the morphology of *B. influenzae* and which failed to grow on plain agar after two or three subcultures were considered to be of the *B. influenzae* group. Approximately three-fourths of the cultures were taken during the months from September, 1932, to January, 1933.

Of the 27 ambulatory cases from the out-patient clinic 11 or 40.7 per cent showed the presence of the influenza bacillus in the nasopharynx. A control group of 17 patients attending the same clinic for other neurologic conditions showed 5 or 29.4 per cent harboring the influenza bacillus. In the hospital 70 patients were studied. Only 13 or 18.5 per cent showed the presence of *B. influenzae* in the nasopharynx. Of 83 control cases from the same wards, 10 or 12.5 per cent were positive. Taken as a whole, out of a total of 97 patients with chronic encephalitis, 24 or 24.7 per cent showed the presence of the influenza

wrote, "Whether an attack of the first (influenza) acts to predispose to an attack of the second in the manner of the predisposing effects of influenza on bacterial affections of the respiratory tract, is another question awaiting close study." He asked further, "Could epidemic encephalitis be merely one manifestation, albeit a severe one, of epidemic influenza? That it is such a manifestation merely has been repeatedly asserted. The relationship has been as assiduously denied as upheld." Wimmer<sup>15</sup> evidently was of a similar opinion when he stated, "Should, now, the first attack be regarded as a true influenza, predisposing the soil for the encephalitic disease, as it has already been suggested by von Economo? Or should this and following attacks be considered as febrile encephalitic relapses. . . ? Or could it possibly be a matter of reinfection?"

Kramer<sup>16</sup> is of the opinion that influenza is one of the factors, many of which are unknown, which are responsible for the epidemic spread of the sporadic cases of encephalitis. Zinsser<sup>17</sup> concluded that a great deal of the evidence supports the view that the coincidence of influenza and epidemic encephalitis may be causally related, one disease in some manner making the individual susceptible to the other.

One feels, therefore, that while the question of the possible relationship of epidemic encephalitis to influenza has been widely discussed and diametrically opposite opinions held by qualified investigators, one must agree with the conclusions of the second report<sup>18</sup> of the Matheson Commission that the question of the etiology of epidemic encephalitis is still unanswerable, and that the relationship of the two diseases requires more intensive study.

In this regard the work of Stewart and Evans<sup>19</sup> is of great interest. In 1930 these workers reported the results of their bacteriologic and clinical studies on patients with chronic encephalitis. They felt that no serious attempts had been made to discover whether there is a relationship between epidemic encephalitis and the Pfeiffer bacillus. To determine this, they studied 144 patients with Parkinsonism. These included patients who gave a history of an antecedent acute encephalitis or influenza. Nasopharyngeal cultures of these patients showed the presence of the Pfeiffer bacillus in 100 per cent of the cases. A control series of 122 ward and out-patients showed the Pfeiffer bacillus to be present in only 45 per cent of the patients. On the basis of these striking results, agglutination tests were made with the same patients. These tests were positive with the Hammett strain of the Pfeiffer bacillus in 100 per cent, the agglutination titers ranging from 1 to 20 to 1 to 60. As a check, the serums of these patients were found to be agglutinated in only three instances by other organisms. Of the 122 control patients only three febrile cases showed agglutination with the Pfeiffer bacillus.

Feeling that their work suggested a relationship between the Pfeiffer bacillus and epidemic encephalitis, the authors prepared a soluble antigen of the Hammett strain of the Pfeiffer bacillus. Sixty-two patients were injected intramuscularly with 0.1 c.c. of a 1 to 10,000 dilution of the soluble antigen every five to seven days for four doses. The dosage was later increased to 0.2, 0.3, 0.4 c.c. for four doses. Improvement, according to the authors, consisted in the reduction of rigidity. The patients felt less stiff, regained the use of the arms and

legs, the posture became more erect, the face more mobile and the speech less monotonous. No febrile reactions were noted. Stewart concluded that 78 per cent of her patients with chronic encephalitis showed definite decrease in rigidity. The tremors either were not affected or were often increased.

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baecillus. Influenza was endemic in this vicinity during these months. Stewart and Evans, unfortunately, do not tell us what time of the year their cultures were taken.

Treatment with the Hammett strain of the Pfeiffer bacillus in the form of the soluble antigen was begun in 1932. At first the dosage recommended by Stewart was employed. When it was found that very little or no improvement was obtained with this dosage, all patients received 0.5 c.c. of the antigen weekly. As far as we could ascertain, there were no local or generalized reactions following the injections. Urine, blood, and temperature examinations during the course of the injections revealed nothing of significance. No untoward effects were ever observed from the injection of 0.5 c.c. of the antigen. At the beginning of the treatment many of the patients had been removed entirely from the medication they had been receiving for the past several months. Following the withdrawal of the medication it was found necessary to put a certain number back on their medication because of the development of difficulty in swallowing, marked weakness, rigidity, or for other symptoms common to this clinical syndrome.

Just before the injections were begun a physical status of each patient was determined by one of us (J. L. A.). This was compared with the condition of the patient at the completion of each series of four injections. Improvement was determined by questioning the patient and by noting his gait, his ability to get about and feed himself, the position of the body, the amount of rigidity in the arms, the intensity of the tremors, and the frequency of the oculogyric crises. The personal equation in evaluating the results was necessarily present, but since all examinations were done by the same individual, it was felt this would be minimized.

Approximately 125 patients with chronic encephalitis received injections of the soluble antigen of the Hammett strain of the Pfeiffer bacillus. Treatment in most cases consisted of a series of weekly injections of 0.5 c.c. of the antigen. In some cases the lack of any improvement and the hardship suffered by the patient by the withdrawal of all other medication caused us to cease using this form of treatment after one or two months. The majority of the patients, however, continued to receive the antigen for twelve to eighteen months.

#### CONCLUSIONS

It is often difficult to estimate the value of any method of treatment in the chronic stage of epidemic encephalitis. A change of medication is often likely to be attended by temporary improvement. In our series we found that those who were bedridden universally failed to receive any benefit from this or from any other form of treatment. A few of the patients, who were up and about and who were being benefited by large doses of drugs (hyoseine hydrobromide gr. 1/50 t. i. d.), maintained their physical level when they were receiving the injections by taking half the amount of the drug. It is difficult to explain this. At the end of treatment with the antigen over a period of eighteen months, we were unable to note any persistent improvement in any of the 125 patients, which

we could attribute to this form of treatment. Occasionally some of the patients thought they could walk with less rigidity, but they soon relapsed into their former state while continuing to receive the antigen.

Our results are in marked contrast to those obtained by Stewart and Evans. These workers reported improvement in 78 per cent of the patients treated by the same method. The material used by them and by us was manufactured and supplied by the same company.\* We feel that our patients have been treated for a sufficient period of time to permit us to judge the efficiency of this form of treatment. Moreover, an attempt to emulate the laboratory work of Stewart and Evans in every detail resulted in a much lower percentage of positive cultures. Possibly the time of year when the cultures were taken might account for some of the discrepancy. In the treatment of the patients it occurred to us that possibly those patients who gave a history of having had the "flu" or the grip might react differently to the antigen of the Pfeiffer bacillus than those who gave a history of an antecedent acute encephalitis. However, we were unable to demonstrate such a difference. One must, therefore, conclude that as far as our results go, there is no relationship between chronic encephalitis and the Pfeiffer bacillus. If one assumes that this bacillus is the etiologic agent of influenza, then we feel that no direct relationship exists between influenza and epidemic encephalitis.

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## COMMENTS ON ANALYSES OF BLOOD CHEMISTRY OF CIRCA FIVE HUNDRED PATIENTS WITH COMMON SKIN DISEASES\*

HERMAN GOODMAN, B.S., M.D., NEW YORK, N. Y.

ABOUT 500 patients were studied on the Out-Patient Service of Dr. Herman Goodman at the Stuyvesant Square, formerly the New York Skin and Cancer Hospital. Blood chemistry examinations were made of this group at the Research Laboratory of the Hospital. Charts were prepared graphically to present the findings, and our comments given here are based on the study of these charts.

### SUGAR

The average blood sugar for 467 patients was 96.5 mg. per 100 c.c. We had 162 patients with acne (of whom 18 had seborrhea, 2 tinea, 1 eczema, and 1 furuncle). The average blood sugar for the acne group was 93.2 mg. which is slightly under the general average. The high of this acne group was 127.4 and the low 69.9. A group of 46 patients diagnosed as seborrheic dermatitis gave us an average of 94.4 mg. of sugar per 100 c.c. The high was 133.0 and the low was 76.4. Urticaria was also slightly below the general average, being 93.4 mg. for a group of 17 patients.

The highest average of sugar was among 23 patients with furunculosis. The high for this group was 218.2 and the low was 81.6. Patients with pruritus, 23 in number, gave an average of 102.4 mg. sugar. The high for this group was 229.4 and the low was 81.6 mg.

The other groups studied, dermatitis, eczema, erythema, pityriasis rosea, psoriasis and tinea, gave averages close to the general average of 96.5 mg. per 100 c.c.

We then relisted the cases, removing all patients with a blood sugar over 120 mg. per 100 c.c. The general average for 454 patients with blood sugar less than 120 was 94.4. The 82 patients with eczema gave an average blood sugar of 96.3 mg. per 100 c.c., which was well within the general average. Acne vulgaris (161 cases) gave an average of 93.0 mg. per 100 c.c. It has been recognized of late that this condition is not associated with the high blood sugar findings, once thought to be an accompaniment of the condition. Only one patient in this large group had a blood sugar over 120 mg. This patient had an associated seborrheic eczema with her acne and the blood sugar was 127.4 mg.

One patient of the group with furunculosis had a blood sugar of 218.2 mg. The average for the remaining 10 patients was 94.3 mg., the high was 110.0 and the low 81.6 mg. This would indicate that one of 11 patients with furunculosis was diabetic. No patient of the 19 with psoriasis had a blood sugar over 120

\*Received for publication, November 12, 1934.

mg. Three patients with tinea had very high blood sugar, 230.8, 194.6 and 187.4 mg., respectively. The average of the 39 tinea patients with less than 120 mg. of sugar was 94.0.

By and large then, the number of frank diabetics among patients seeking aid for skin conditions is probably no greater than in the general run of the same group, social, racial, etc., without skin affections. In all, seventeen patients had a blood sugar greater than 120 mg. per 100 c.c. We have already mentioned the patient with acne and seborrheic eczema (127.4); the patient with furunculosis (218.2); the three patients with tinea (230.8, 194.4, 187.4); and now list the remaining patients as: one with pruritus (229.4), four patients with eczema (198.6, 120.4, 120.4), one each with the following: stomatitis (200.0), tertiary syphilis (149.9), erythema (152.5), seborrheic dermatitis (133.0), herpes (130.2), dermatitis (124.6), and ichthyosis (120.4).

#### SODIUM CHLORIDE

The sodium chloride content of the blood was estimated in 466 patients. The general average was 471.6 mg. per 100 c.c. The highest average was found among 23 patients with pruritus, and it was 479.0 with a high of 515.8 and a low of 421.0 mg. per 100 c.c. The lowest average for sodium chloride was among the 11 patients with furunculosis and here it was 467.4 with a high of 483.8 and a low of 446.0 mg. per 100 c.c. The 162 patients with acne gave an average of 469.4 mg. per 100 c.c. The high for this acne group was 515.8 and the low was 420.1 mg. per 100 c.c.

The patients with eczema, 85 in number, gave an average blood sodium chloride of 472.4 mg. per 100 c.c. Nineteen psoriasis patients gave an average of 475.3 mg. of salt per 100 c.c. Other groups, as dermatitis, pityriasis rosea, tinea and urticaria, gave findings close to the general average.

By and for itself, the estimation of the salt and the analysis of the findings limited to the salt content gave us little practical information. On the other hand, a study of the report of the salt content of the blood with the accompanying sugar findings gave us some information. We grouped the blood chemistry findings of 514 patients and arranged them so as to compare the salt and sugar estimations. The average sodium chloride content was 475.5 mg. per 100 c.c. and the average blood sugar was 96.7 mg. per 100 c.c.

#### SALT AND SUGAR IN THE BLOOD

The exciting find we made was that as the blood chloride went up, the accompanying blood sugar went down. For example: we had 17 patients with blood chloride of less than 450.0 mg. per 100 c.c. The high of this select group was 441.8, the low was 408.5, and the average was 429.4. The sugar average for this group was 110.2 mg. per 100 c.c.

We next took a group of 25 patients with an average blood chloride of 446.5 mg. per 100 c.c. The high for this group was 450.1 and the low was 441.8. The blood sugar for this group was 103.2 mg. per 100 c.c. We next selected the cases in groups of fifty with the salt content graduated upward. As the salt averages went up, the sugar content averages went down. It was

not exactly mathematical, but when we reached the average blood chloride of 498.4 for a group of fifty patients with high 507.5 and low 495.0, we found that the blood sugar was 92.9 mg. per 100 c.c.

Now, we thought, if the blood sugar apparently goes down as the salt goes up, what will the figures show if taken from the findings of the sugar instead of the salt? So we rearranged our cases once more. And we find that as the average blood sugar goes down, the average blood chloride goes up. For example, we had 17 patients with blood sugar over 120 mg. per 100 c.c. The blood chloride for this selected group was 455.1 mg. per 100 c.c. The next group of 25 patients, with blood sugar between 110 and 120 mg. per 100 c.c., had an accompanying blood chloride of 475.0 mg. per 100 c.c. Again, we found that the sliding scale was not exactly mathematical, but if we jumped to a group of 50 patients with an average blood sugar of 83.4 (high 85.5 and low 80.2), we find that the blood chloride was 479.8 mg. per 100 c.c.

We check back with our list of patients with high blood sugar and find that the patient with pruritus who had 229.4 mg. of sugar per 100 c.c. of blood had only 421.0 mg. of sodium chloride. The patient with furunculosis who had 218.2 mg. of sugar per 100 c.c. of blood had 446.0 mg. of salt per 100 c.c. The patient with stomatitis with a blood sugar of 200 had 450 mg. of salt. A patient with eczema who had 198 mg. of sugar had 450.1 of salt. One patient with tinea who had a blood sugar of 187.4 gave a blood chloride of 433.5. Of course, this comparison was not always mathematical. If it had been, we would be concerned that the figures were not factual. For example, a patient with tinea who had a blood sugar of 230.8 had a blood salt of 454.3. Another patient with tinea had a blood sugar of 194.4 and a blood chloride of 462.7 mg. per 100 c.c. In both these instances, the blood sugar was much over the average of 96.5 and in turn the blood chloride was much below the average of 471.6. In the above list, we have accounted for the highest 10 of the 17 patients with blood sugar of more than 120 mg. per 100 c.c.

We would turn next to a tabulation of 14 patients who gave us a sodium chloride content of less than 441.0 mg. per 100 c.c. There were three patients of these 14 who had blood sugar of more than 120 mg. per 100 c.c., and two other patients close to it (119.0 and 117.7).

The other nine patients had a low salt content without accompanying high blood sugar; four of these had 437.7 mg. of salt per 100 c.c. The lowest salt content was in a patient with seborrheic eczema whose blood sugar was 81.6 mg. We find listed a patient with pemphigus showing salt content of 437.7 and a sugar of 92.0 mg. per 100 c.c. This is of some interest in the proposed etiology of this strange disease.

Another point of interest is that six patients in this list of low salt content (less than 441.0 mg. per 100 c.c.) were diagnosed *aene vulgaris*. Not one had a blood sugar above 120 mg. per 100 c.c. We did not recognize the importance of this until much later, and hence are unable to report exactly what clinical type of *aene vulgaris* is represented by these six patients.

#### UREA NITROGEN

We learned nothing of startling import by our analyses of the findings of urea nitrogen in the blood of the 466 patients studied. The general average

was 13.5 mg. per 100 c.e. All the groups studied, aene, dermatitis, eezema, erythema, furunculosis, pityriasis rosea, pruritus, psoriasis, tinea, and urticaria were well within this general average. Furmenlosis, for example, was low with 13.0 and pruritus was high with 14.5.

#### URIC ACID

We cannot report anything unusual in the findings for uric acid. The general average for the 466 patients tested was 2.55 mg. per 100 c.e. The low was 2.07 for pityriasis rosea. The high was 2.90 for psoriasis.

#### INFLUENCE OF AGE OF PATIENT ON BLOOD FINDINGS

It seemed worth while to recast our findings according to the age of the patient as well as according to the different ingredients of the blood studied.

#### INFLUENCE OF AGE OF PATIENT ON BLOOD SUGAR

We estimated the blood sugar on 514 patients and found the general average to be 96.7 mg. per 100 c.e. All the groups less than forty years of age averaged less than this general average. For example, 111 patients were between twenty-one and twenty-five years of age. The average blood sugar for this group was 92.1. The group between forty-one and fifty years had 63 patients. The average for this group was 108.4. All the groups of patients more than forty years of age had blood sugar averages more than 105.6 mg. per 100 c.e.

At first sight then, it would seem that what we are stating is that there must be more diabetics among the higher age groups. But we recast our findings once again and remove all blood sugar above 120 mg. per 100 c.e. In general, the average drops from 96.7 to 94.7 as the 17 patients with blood sugar over 120 are deleted. But the swing upward of the blood sugar average with increasing age is still noted. It happened that the group of 111 patients between twenty-one and twenty-five had no blood sugar above 120 so its average remains 92.1. The age group forty-one to fifty lost 8 patients with blood sugar above 120. The average for the 55 patients remaining was 101.3 mg. per 100 c.e., the highest recorded average. The patients more than forty-one had an average of 100 mg. per 100 c.e., which is higher than the general average for all age groups of 94.7 mg. per 100 c.e.

#### INFLUENCE OF AGE ON SODIUM CHLORIDE IN THE BLOOD

The general average of sodium chloride in the blood was 475.5 for the 514 patients studied. The averages for the various groups held quite close to this general average. The highest average for the age groups was 478.7 for the ages thirty-one to forty. The lowest salt average was 468.7 for the age group fifty-one to sixty. In a way, the conduct of the salt holds to our former findings, for the older people, that is above fifty-one years old, averaged the lowest salt and the highest sugar findings. Again this was not mathematical, and we are glad that it was not so for it would smack too much of window dressing.

#### INFLUENCE OF AGE ON UREA NITROGEN IN THE BLOOD

The general average of urea nitrogen for 502 patients was 13.6 mg. per 100 c.e. The lowest age group was that between twenty-one and twenty-five.



There were 111 patients in this group and the urea nitrogen averaged 12.8 mg. per 100 c.c. The highest average was for the small group of 15 patients more than sixty years of age with an average of 15.7. But the next highest was for the youths of our series, patients under fifteen years of age, of whom there were 22 who had an average of 14.8 mg. of urea nitrogen per 100 c.c. of blood.

#### INFLUENCE OF AGE ON URIC ACID IN THE BLOOD

The general average of uric acid for 500 patients was 2.62 mg. per 100 c.c. The lowest group was between sixteen and twenty years of age. There were 82 patients in this group and the average was 2.39 mg. of uric acid per 100 c.c. The highest average was in the group of 45 patients between the ages of fifty-one and sixty. The uric acid average was 2.93 mg. per 100 c.c.

#### SUMMARY

We have commented upon the results of blood chemistry studies made on about 500 patients with various skin disorders. We note that blood sugar is high in furunculosis as an average because one patient was a frank diabetic with a very high blood sugar. We offer, then, the process of consideration of our average finding with all frank diabetics removed. Then, the sugar findings are without any particular significance by themselves.

The analyses of the salt findings were not significant by themselves, either. But we disclosed a relationship between salt and sugar which we thought very significant; namely, that by and large as the sugar averages went down, the salt averages went up. This relationship, the antagonism between sugar and salt, was not absolute. It was fairly constant. The converse was also noted: as the average salt content of the blood increases, the average blood sugar decreases. We do not offer any reason for this reciprocal arrangement. We are certain that it is not fiction nor bookkeeping.

On the basis of the findings of antagonism between sugar and salt in the blood, and the accidental find that saline intravenously relieved a pustular acne (which we first considered an acne due to ingestion of bromides), we have experimented with salt solution intravenously and locally into pustules, with gratifying results. The further possibilities of this form of therapy are suggested.

We found nothing exciting in the blood urea nitrogen or uric acid among our patients.

We did find that the content of sugar gradually went higher with increasing years, and that this was true whether we deleted frank diabetics or left them in the analysis. Older patients retain sugar, urea nitrogen, and uric acid. They do not retain more salt, but tend, as the sugar goes up, to have less than the average saline content of the blood.

We believe that this method of presenting blood findings for a series of about 500 patients, seeking aid for dermatologic ailments, has not been previously presented.

## EXPERIMENTAL STUDIES IN SO-CALLED AGRANULOCYTIC ANGINA\*

EFFECTS OF TOXIC PRODUCTS OF CERTAIN BACTERIA RECOVERED FROM THE STOOL  
AND BLOOD OF THE HUMAN BEING UPON THE LEUCOCYTES OF ANIMALS

WILLIAM H. HARRIS, M.D., AND HERBERT J. SCHATTENBERG, M.D.,  
NEW ORLEANS, LA.

THIS unusual clinical entity was first described by Schultz<sup>1</sup> in 1922 and called by him agranulocytic angina. The accuracy of the name had been questioned in various reports, and other names deemed more suitable have been applied. The disease has been called angina agranulocytic (Friedman), mucositis necroticans agranulocytica (Weiss), granulocytopenia (David), and malignant neutropenia (Schilling). A solution of such a discussion may perhaps be reached by terming this condition "the Schultz syndrome," especially as thus far the indications are that its causation is of a protean rather than a specific nature. The leucocytic picture present is generally a granulocytopenic leucopenia, whereas the associated clinical and other laboratory findings may at times vary. A destructive throat lesion is, however, usually present. Replete and recent compilations of the literature are given by Kraeke<sup>2</sup> and in the editorial symposium section of the *International Medical Digest*.<sup>3</sup> The study of the bone marrow and a complete review of the literature on this phase of the disease recently have been presented by Jaffé.<sup>4</sup>

In experiments relating to the cause of the Schultz syndrome various observations have been reported. Dennis<sup>5</sup> has shown that when various virulent microorganisms are enclosed in parchment capsules and introduced into the peritoneal cavity of rabbits, in some instances reduction of the total leucocytes and granulocytes is produced by the absorption of the permeating toxins. The microorganisms employed by him were not derived from the human disease, excepting a *B. proteus* which did not cause any marked changes. Kraeke<sup>6</sup> reports that various microorganisms recovered from throat lesions and other areas fail to produce granulocytic neutropenia when injected into animals. He produced the blood picture and, in some instances, throat lesions by the injection of benzene with olive oil. It had been previously shown by Selling both clinically<sup>7</sup> and experimentally<sup>8</sup> that benzene is capable of producing in certain patients a granulocytopenia with purpura and hemorrhages, and in the experimental animal, reduction of the leucocytes to 200 cells per c.mm. with but little alteration of the erythrocytes. In this

\*From the Department of Pathology, School of Medicine, Tulane University.  
Received for publication, November 19, 1934.

Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

connection, because of a supposed benzene content, much interest has recently arisen as to the probable causal relationship of the various barbital derivatives. It must be realized, however, that some of these drugs considered as causes actually contain no benzene factor, and furthermore that other drugs of the benzene group in very general use are not incriminated.

#### MATERIALS

Our materials were procured from a fatal chronic case occurring in a child, eleven years old, in whom the primary throat lesion, diagnosed as Vincent's angina, had disappeared but a marked blood dyscrasia was persistent. The patient presented boils and two large abscesses and revealed a positive *Staphylococcus aureus* blood culture several days prior to death.

As far as ascertained by us, the possible causal relationship of certain of the intestinal flora of such cases has not been investigated. We accordingly carried out a bacteriologic investigation of the stool specimens, and recovered cultures of *B. enteritidis*, *B. welchii*, and *Streptococcus hemolyticus*. In the experiments herein reported, these cultures obtained from the stools and also the culture of *Staphylococcus aureus*, procured from the blood culture together with toxic products obtained from these isolations, were employed. These were injected by various routes into animals to ascertain their effects, especially upon leucocytes. Seventy-nine guinea pigs and sixteen rabbits were used. The leucocytic counts of rabbits as found by other observers<sup>9, 10, 11</sup> and ourselves<sup>12</sup> reveal total counts of from 8,000 to 10,657 cells per c.mm. and a differential granulocytic count of 35.4 per cent to 54.0 per cent. These counts in the guinea pig show the usual established variation of from 6,000 to 12,000 leucocytes per c.mm. and granulocytes of approximately 35 per cent.<sup>13</sup>

#### EXPERIMENTAL

*B. enteritidis*.—Six guinea pigs were injected, two subcutaneously, two intraperitoneally, and two intracardially with 1 c.c. each of a saline suspension, containing approximately 2.5 billion bacteria per cubic centimeter of this strain of *B. enteritidis* and all died in from twenty-four to seventy-two hours. Although repeated counts were made even up to shortly before death, no evidences of reduction of the total leucocytes or granulocytic counts were produced but on the contrary a neutrophilic leucocytosis prevailed.

Three guinea pigs were fed daily with oats contaminated with the stools of the human being containing *B. enteritidis* and over the food of 3 additional guinea pigs, suspensions of the recovered culture of this bacillus were poured. After six weeks of observation, including repeated leucocytic counts, no appreciable effects of these feedings were noted.

Four guinea pigs were injected with the filtrate of broth cultures of the *B. enteritidis*, two intracardially and two intraperitoneally. The resultant leucocytic counts showed a total and granulocytic increase.

Since such direct introductions of the *B. enteritidis* and culture filtrates failed to produce granulocytic leucopenia, it was decided to employ the toxic products that might be procured through the in vivo method as used by one

of us (Harris) in the experimental production of the lesions of typhoid fever,<sup>14, 15</sup> with a concurrent leucopenia. In this method fatal peritonitis is primarily produced in the guinea pig and the exudative material and washings of the peritoneal cavity are collected and filtered through a Berkefeld or Seitz-Wertz filter. The purpose of this procedure is to obtain the toxic factors that may result from the invader and host conflict. The supply pigs receive 2 c.c. intraperitoneally of a heavy suspension of the living *B. enteritidis* suspension. Death from the induced peritonitis occurs in twelve to twenty-

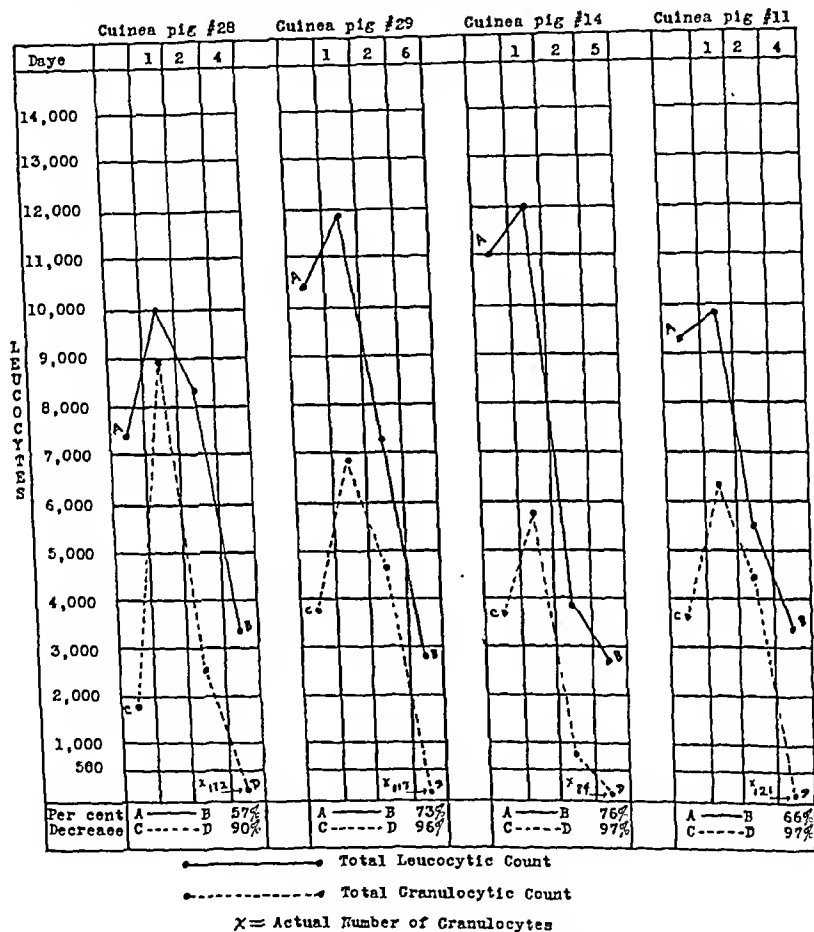


Chart 1.—Effects of toxic products of *B. enteritidis* upon leucoytes.

four hours. Fifteen guinea pigs were injected with the toxic materials procured through this method. The toxic Seitz-Wertz filtrate was administered in doses of from 1 to 5 c.c. subcutaneously, intraperitoneally, and intracardially to different groups of animals. The intraperitoneal and intracardial routes and the larger doses were more effective. In all animals injected with the filtrate, a distinct lowering of the total leucocytic counts with a proportionately greater reduction of the granulocytes was observed. The period at which the greatest decline occurred averaged seventy-two hours with ordinarily an increment in the first twenty-four hours. As a series, the intra-

cardiac route yielded the most marked leucopenia and granulocytopenia. Representative changes produced in the leucocytic counts of four animals thus inoculated are shown in Chart 1. Reductions of from 57 per cent to 76 per cent of the total white cell count and of from 90 per cent to 97 per cent of the granulocytes are demonstrated. Four guinea pigs were injected intracardially with toxic peritoneal filtrate procured from a stock strain of *B. enteritidis* and the inhibitory effect upon the granulocytes and leucocytes

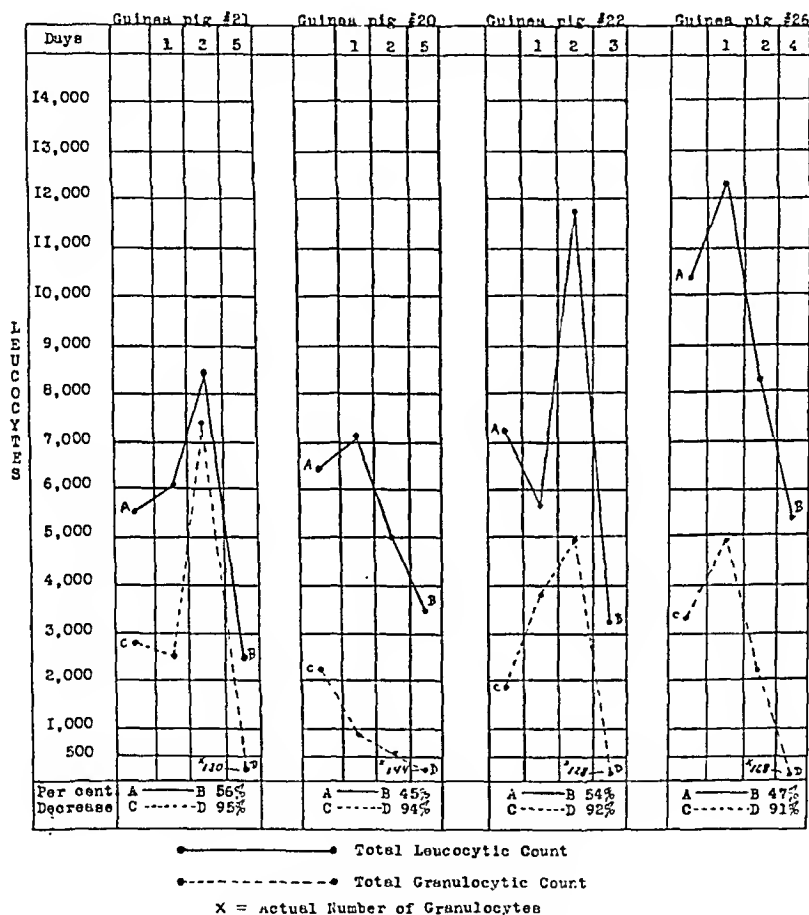


Chart 2.—Effects of toxic products of *B. typhosus* upon leucocytes.

as a whole was much less marked. This result is attributed to the fact that this culture had been carried in stock for over four years.

For comparison, an old stock culture of *B. typhosus* that was known to depress leucocytes by its in vivo toxic products, was employed. Twelve guinea pigs were treated in the same manner with toxic peritoneal exudate filtrates of *B. typhosus* peritonitis as were those with the enteritidis and, in general, similar results were obtained. The results of four guinea pigs injected intracardially with this material are shown in Chart 2. The culture used in these injections had been in artificial cultivation for about five years. When this

culture was primarily recovered from the patient and further passed through several guinea pigs, the resultant leucopenia was much more marked and the gross and histopathology of typhoid fever were produced.<sup>15</sup>

*B. welchii*.—Upon the first series of animals consisting of six full-grown rabbits, the recovered cultures of *B. welchii* were employed in the following manner: The whey portion of litmus milk cultures was filtered through a Seitz-Wertz filter and the toxic filtrate retained. It is to be appreciated that various protein products aside from the bacterial toxin were contained in the

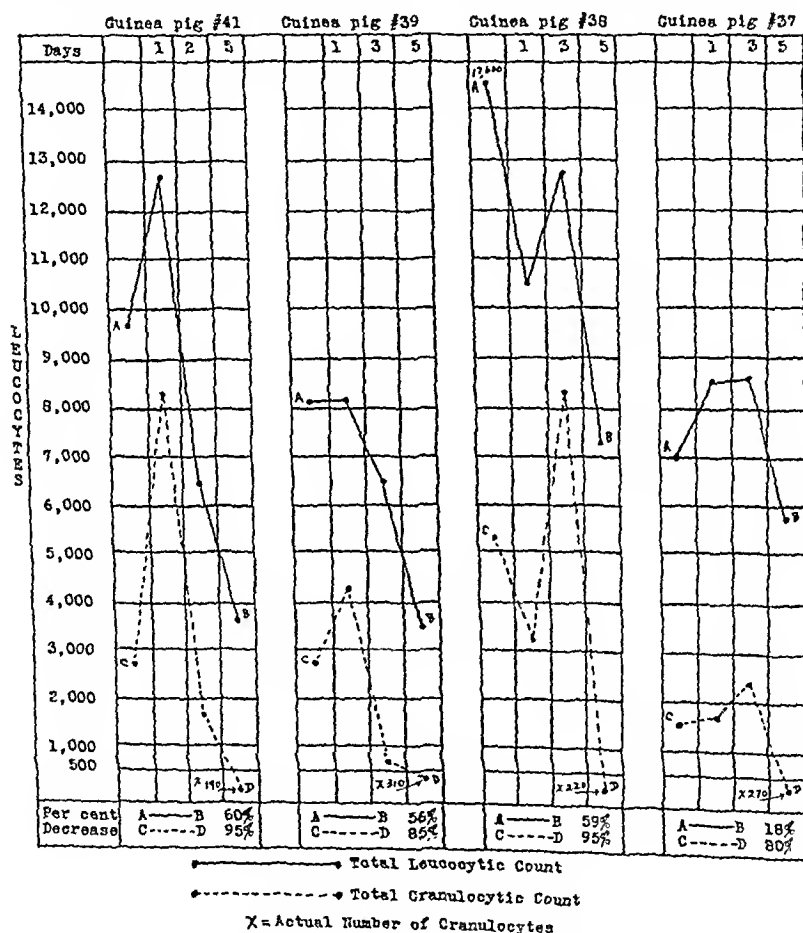


Chart 3.—Effects of toxic products of *B. welchii* upon leucocytes.

filtrate. This filtrate in amounts of from 2 to 10 c.c. was introduced daily for two weeks into the stomach through a tube. No effects upon the general well-being of these animals or the leucocytic counts were noted. The living culture of this microorganism was administered through a tube into the stomach of four rabbits. These animals, likewise, showed no changes in their physical condition or in the frequent leucocytic observations that were made.

The toxic filtrate of *B. welchii* was next administered into two rabbits intravenously and nine guinea pigs intracardially. Each of the two rabbits was given two doses intravenously of 1 c.c. at six-day intervals. The total

leucocytic count was primarily increased following these injections with a corresponding increase of the granulocytes, but after seventy-two hours the total count returned to normal. The granulocytes, however, were reduced from an average of approximately 40 per cent to amounts as low as 5 per cent following a primary rise, but later returned to normal. The guinea pigs injected intracardially with this toxic filtrate in doses of more than 0.5 c.c. usually succumbed within twenty-four hours. In the animals inoculated with 0.25 to 0.5 c.c. the leucocytic and granulocytic curves were the same as those of

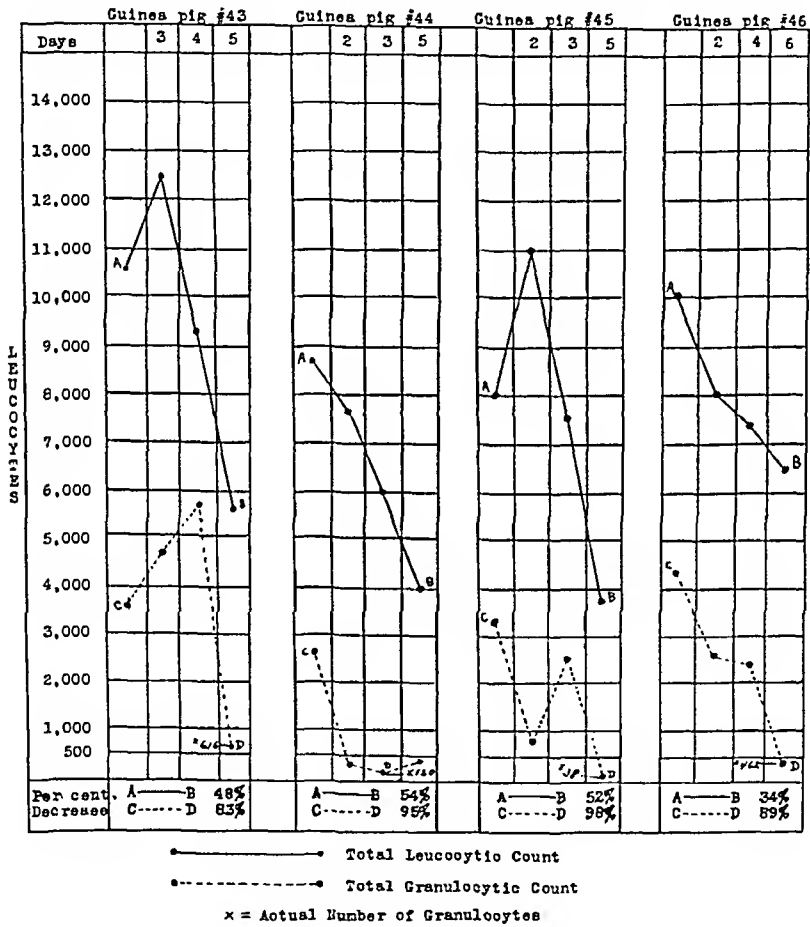


Chart 4.—Effects of toxic products of *Streptococcus hemolyticus* upon leucocytes.

the rabbit, i.e., primary rise of both with subsequent return of the total count but diminution of the granulocytes to between 5 and 8 per cent. Four guinea pigs were injected intraperitoneally with 3 to 5 c.c. of the welchii filtrate, and these animals yielded in certain instances marked reductions of both the total leucocytic and granulocytic cells. The effects produced upon the leucocytes are shown in Chart 3. Reductions of the total white cell counts of from 18 per cent to 60 per cent are seen, whereas the granulocytic reductions were as much as 95 per cent.

*Streptococcus Hemolyticus*.—The inoculation of this culture subcutaneously, intraperitoneally and intracardially yielded neutrophilic leucocytosis. Filtrates of broth cultures introduced into four guinea pigs did not reduce the leucocytes or granulocytes. In vivo prepared toxic products were obtained by intraperitoneal injections of this microorganism into the guinea pig. As it was only slightly pathogenic for this animal, two injections of heavy suspensions were given at seventy-two-hour intervals when peritonitis and death ensued. The Seitz-Wertz filtrate of the peritoneal exudate and washings was

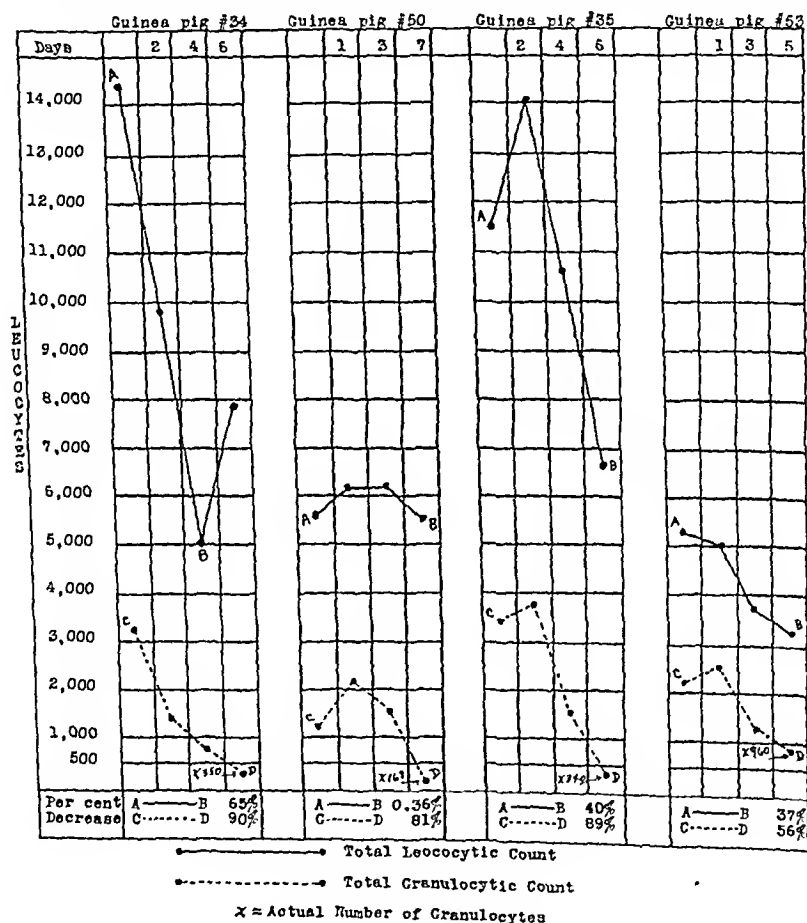


Chart 5.—Effects of toxic products of *Staphylococcus aureus* upon leucocytes.

injected intraperitoneally into two guinea pigs, Nos. 45 and 46, in doses of 5 c.e. and intracardially into guinea pigs Nos. 43 and 44, each receiving 3 c.e. Considerable reduction of the total leucocytic count with marked reduction of the granulocytes was produced as shown in Chart 4. Again, it is shown that both marked reductions of the total leucocytes (54 per cent) and granulocytes (98 per cent) were produced.

*Staphylococcus Aureus*.—Injections of suspension of the culture of *Staphylococcus aureus* subcutaneously into two guinea pigs and intraperitoneally into two other such animals produced neutrophilic leucocytosis. Rabbits 2,



3, and 5 were inoculated in the following manner: Rabbit 2 received 2 c.c. of the living suspension in the peritonsillar structures which eventually produced a local necrosis in this area. Frequent counts revealed only a neutrophilic leucocytosis. In Rabbit 3 the upper tracheal mucosa was scarified with the hypodermic needle and 1 c.c. was injected in and about the eroded area. Neutrophilic leucocytosis ensued. Rabbit 5 was given 5 c.c. of melted agar subcutaneously. After the agar became semisolid, 1 c.c. of the culture

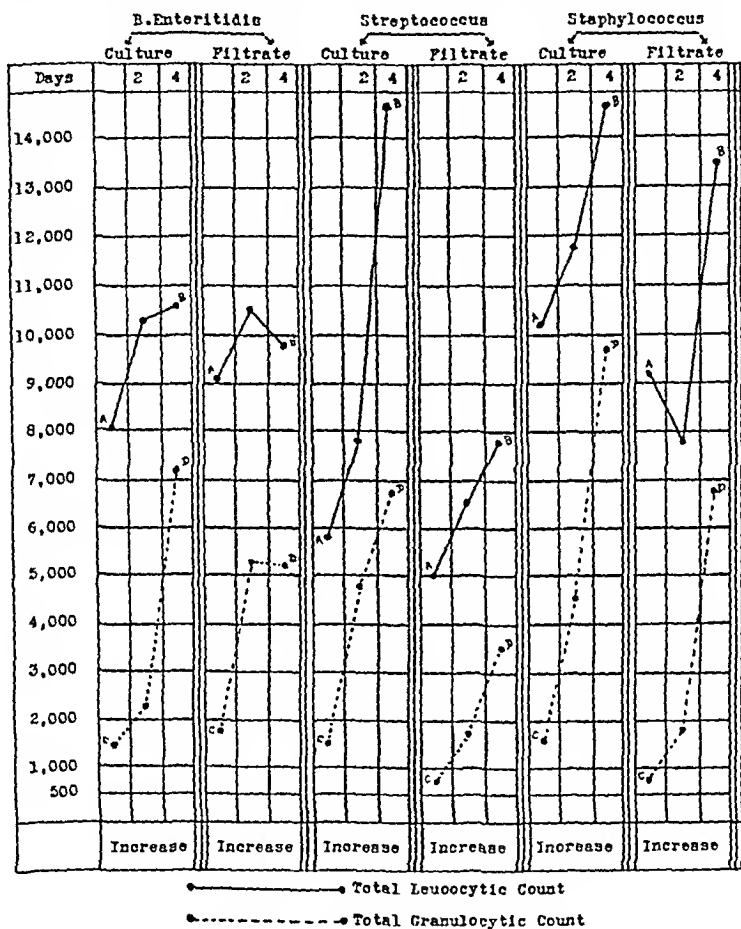


Chart 6.—Control animals injected with living culture or culture filtrate.

of *Staphylococcus aureus* was introduced into the mass. Only a slight rise of the total leucocytes and granulocytic cells was noted upon repeated counts.

Four guinea pigs were injected with the filtrate of broth cultures of this staphylococcus, two intracardially and two interperitoneally. These animals revealed only neutrophilic leucocytosis.

An in vivo toxic filtrate prepared as in the previous instances was obtained from this staphylococcus and injected intraperitoneally and intracardially in doses of 3 c.c. into six guinea pigs, three for each route. Some reduction of the total leucocytic count and marked reduction of the granulocytes were produced (see Chart 5). It is of interest to note that one of these

animals developed an erythroblastic anemia, indicating a disturbance of this portion of the hemopoietic system as well as that of the myeloblastic centers.

As has been heretofore stated, the direct injections of the employed cultures of *B. enteritidis*, streptococcus and staphylococcus and their broth culture filtrates failed to produce granulocytopenia. In Chart 6 is shown a number of these animals injected with the culture and culture filtrates which serve as controls in comparison with the animals injected with the in vivo prepared toxin.

In general, it may be stated that repetition of the injections of in vivo prepared toxin into the same animals produced a duplication of the results described. After several such injections, the majority of the animals sickened and died. In these animals pneumococci and terminal invaders were obtained from hearts' blood.

Smear preparations of the bone marrow of those animals demonstrating marked granulocytic depression showed, as a whole, degenerative changes in the nuclear portion of the myeloid cells. Different findings have been noted by various observers, a compilation of which has been set forth by Jaffé.<sup>4</sup>

#### DISCUSSION

From the results presented herein and the work of others, it can be appreciated that various microorganismal poisons will produce the marked leucocytic changes occurring in the Schultz syndrome or so-called agranulocytic angina as found in man. The continuation of such leucocytic depressions eventually leads to a morbidity of the animal with lowered resistance and secondary infection as frequently noted in the human being.

Whether such parallelisms represent a true reproduction of the human disease is naturally subject to question and, no doubt, will remain so unless a specific causal agent is later discovered. In this connection, it is difficult to understand, if a heterologous group of toxic factors are responsible for the disease, why it does not occur more frequently. It might be surmised that this rather unusual phase of certain toxins occurs infrequently, or, as has been suggested,<sup>10</sup> a rather rare allergic phenomenon is displayed.

In the human being, it is rather remarkable that a secondary invasion of *Staphylococcus aureus* occurs so frequently unless the lowered neutrophilic resistance and wide distribution of this microorganism is considered responsible. In connection with such staphylococcal invasions occurring both locally and at times systemically, it is of note that such a pyogen with actual production of pus or an accumulation of neutrophilic granulocytes should be accompanied by a systemic granulocytic depression or at times absence of such cells in the circulating blood stream. Perhaps the "shifting of the vascular bed" as indicated by Garrey<sup>11</sup> might explain their source. Kracke,<sup>2</sup> however, has demonstrated that the blood removed from the veins in this disease demonstrates the same marked leucocytic and granulocytic depression.

Dennis<sup>5</sup> considers that the absorption of toxic material without bacterial invasion into the tissues may cause this condition. He, therefore, employed the intraperitoneal parchment capsules containing the microorganism to dem-

onstrate this possibility. In the present experiments, with the exception of the *B. welchii* toxin, the liberated toxic moieties were obtained from filtrates of the peritonitis exudates. It is considered that in this in vivo encounter of microorganisms and host there is liberated a toxic substance that is not ordinarily obtained in vitro. Such in vivo toxins can be readily obtained, and while their activity when injected is transitory, repeated injections will serve as a sufficiently continuous source.

### CONCLUSIONS

1. From the observations made herein, it is evident that marked depression of the total and granulocytic leucocytic counts can readily be produced by the toxic moieties of various bacteria. The toxin of bacteria of this disease, obtained from other sources than that of the human being, will likewise cause such reductions. Recent isolations in which the virulence is exalted produce more distinctive results.

2. For the bacteria herein employed, a toxic moiety obtained only from an in vivo source is effective, with the exception of *B. welchii*. Repetitions of such toxic injections into animals reduce their resistance, and they become sick and die, frequently with terminal infections.

3. Relative to the etiology of agranulocytic angina or the Schultz syndrome, these experiments would indicate that the causation is of a protean or varied nature.

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# A STUDY OF THE COMPARATIVE VALUE OF THE LÖWENSTEIN METHOD AND THE DIRECT CULTURE OF WHOLE BLOOD IN DETECTING TUBERCLE BACILLI IN THE BLOOD STREAM\*

LUCY MISHULOW, B.A., MORRIS SIEGEL, M.D., BELLA SINGER, M.D.,  
MILDRED MELMAN, B.A., AND MARIE ROMANO, B.A., NEW YORK, N. Y.

TUBERCULOUS bacillemia has been the subject of a great deal of study, especially since Löwenstein and his associates<sup>1 a, b, c, d</sup> reported a high percentage of positive results not only in various tuberculous infections, but also in apparently nontuberculous conditions such as rheumatic fever, polyarthritis, and dementia precox. Löwenstein devised a special method of culturing the blood for tubercle bacilli<sup>2</sup> in which the hemoglobin is removed from the blood before the specimen is cultured. He stressed the importance of completely removing the hemoglobin as he believed that it interferes with the growth of the tubercle bacilli. Many have attempted to confirm Löwenstein's work but only a few reported successful results. Most of the workers were able to obtain only a comparatively small percentage of positive cultures in tuberculous cases, and some had entirely negative results or obtained a few saprophytic acid-fast cultures.<sup>3 to 15</sup> According to Löwenstein, however, this failure to obtain positive results may have been due in part to incomplete removal of the hemoglobin.<sup>1 d</sup>

One of us<sup>16</sup> found in connection with other investigations that human and bovine tubercle bacilli grew freely and rapidly on the Bordet-Gengou medium which contained about 30 per cent of horse blood. Also that human tubercle bacilli grew freely when the whole blood of infected rabbits was cultured on the Bordet-Gengou and on egg medium without any preliminary treatment.<sup>17</sup> Dreyer and Vollum<sup>18</sup> obtained positive blood cultures from rabbits and guinea pigs by inoculating whole blood into plain or glycerin bouillon. Alexander<sup>19</sup> obtained positive cultures from tuberculous mice by growing the whole blood on the Bordet-Gengou medium.

Since the hemoglobin of animal blood apparently did not interfere with the growth of tubercle bacilli, it seemed desirable to determine whether human blood differs in this respect from animal blood. If in the search for tubercle bacilli whole blood could be cultured directly, it would be very much simpler and consume much less time than culturing according to Löwenstein's method. With this object in view we carried out the following investigation:

Specimens of blood were obtained from tuberculous patients at the Sea View Hospital. The amount of blood cultured varied between 4 and 14 c.c. depending upon the case with which the blood was obtained. In order to make

\*The "Whole Blood Culture" was carried out at the Research Laboratory of the New York City Department of Health under Director Dr. Wm. H. Park, and the "Löwenstein Culture" was carried out at the Sea View Hospital Pediatric Department under Dr. Bela Schick.  
Received for publication, November 7, 1934.

the two methods directly comparable each specimen was divided into two equal parts. One part was mixed with sterile 0.4 c.c. of 10 per cent sodium citrate to prevent coagulation and was used for the direct culture, and the other part was cultured by the Löwenstein method.<sup>2</sup>

#### WHOLE BLOOD CULTURE METHOD

As soon as the citrated blood was received at the laboratory, it was inoculated by means of a pipette on the surface of Bordet-Gengou and of Congo red egg\* mediums in Petri plates, using 6 to 10 plates for each specimen. Then to each plate was added about 1 c.c. of sterile distilled water in order to facilitate the spread of the blood thus hemolyzed, and the plates were tilted back and forth until the blood was evenly distributed over the whole surface. They were then incubated at 37.5 to 38° C. for two to three days or until the excess moisture had evaporated. Care was taken not to allow the plates to become too dry. After this preliminary incubation the plates were sealed with three-fourth-inch Scotch photographic tape and incubated for two months. During the period of incubation the cultures were examined by means of a low power microscope once a week to detect an early growth. Any growth that even remotely resembled colonies of tubercle bacilli was examined by smear and in some instances by subculture.

#### LÖWENSTEIN'S METHOD

The blood was mixed with 2 to 3 c.c. of sterile 10 per cent sodium citrate in centrifuge tubes of 50 c.c. volume and well shaken to prevent clotting. About 30 c.c. of sterile distilled water was added; the contents were shaken and centrifuged for twenty minutes at about 2,000 revolutions per minute. The supernatant fluid was decanted; the sediment was well shaken and again washed with about 30 c.c. of sterile water, and centrifuged for twenty minutes at about 2,000 revolutions per minute. The supernatant fluid was decanted, leaving behind a small grayish sediment. The sediment was thoroughly broken up with a pipette (3 mm. bore) into which all the material was drawn and then spread over the surface of 3 or 4 tubes of Löwenstein's medium. The tubes were well sealed at once with sealing wax, and allowed to lie horizontally for twenty-four hours. Then the tubes were placed upright in the incubator at 38° C. for two months. If no growth was macroscopically visible at this time, a smear was made of the edges, bottom, and surface of the medium. If the result of the microscopic examination was negative, the surface of the tubes was scraped into a small amount of distilled water. This was centrifuged for one-half hour and the sediment again examined by smear. In all microscopic examinations, the Ziehl-Neelsen method of staining was used and the whole surface of the smear was examined by means of a mechanical stage.

We have examined in this manner 98 specimens of blood from 63 tuberculous patients and 2 specimens from a case of actinomycosis of the jaw. The results obtained are briefly summarized in Table I.

Only four positive cultures were obtained in this series and all of them in the group of far advanced pulmonary tuberculosis where the patients were

\*Löwenstein's Congo red egg medium.

TABLE I

RESULTS OBTAINED BY DIRECT CULTURE OF WHOLE BLOOD AND BY LÖWENSTEIN'S METHOD IN DETECTING TUBERCLE BACILLI IN THE BLOOD STREAM

DIAGNOSIS	NO. OF CASES	AGE	NO. OF CASES	NO. OF SPECIMENS	POSITIVE FOR TUBERCLE BACILLI	
					DIRECT CULTURE WHOLE BLOOD	LÖWENSTEIN'S METHOD
Advanced pulmonary tuberculosis (weak or critical)	46	20 to 63 yr.	28	36	1 (Mac.)†	0
		6 to 18 yr.	11*	14	0	1 (Mic.)‡
		9 mo. to 4 yr.	7	22	2§ (Mac.)	2§ (Mac.)
Pulmonary and bone tuberculosis (critically ill)	10	22 to 40 yr.	8	11	0	0
		17 yr.	1	3	0	0
		1½ yr.	1	1	0	0
Bone tuberculosis (pre- and post-operative)	6	22 to 36 yr.	6	9	0	0
Mesentery, cervical and intestinal tuberculosis	1	2 yr.	1	2	0	0
Actinomyces of the jaw	1	3 yr.	1	2	0	0
Total			64	100	3	3

Total number of cases positive by both methods = 4

\*In this group there were isolated two cultures of nonpathogenic acid-fast bacilli. In Case L14 a chromogenic saprophyte was obtained in the portion of blood that was treated by Löwenstein's method, and in Case L55 a single colony of a chromogenic acid-fast bacillus was found on the portion that was cultured directly on the Bordet-Gengou medium.

†Macroscopic growth.

‡Microscopic growth (see text).

§Both patients died after the last specimens were taken (See Table II).

either weak or critically ill. In two instances the cultures were positive by both methods; in the third, the "whole blood" culture was positive and the "Löwenstein" culture negative; and in the fourth, the "whole blood" was negative and the "Löwenstein" culture gave a doubtful positive (see Table II). In one of the cases in this series blood was obtained two and one-half hours before delivery and again five minutes after expulsion of the placenta in order to determine whether there was any blood invasion during labor. The blood of this patient remained consistently negative by both methods although a total of 5 specimens of blood were examined on four successive days. In one case of bone tuberculosis, blood was taken one hour before an operation on the spine and again one-half hour after the operation; both cultures gave negative results.

In two instances chromogenic, saprophytic acid-fast bacilli were isolated from the blood cultures. In Case L14 the saprophyte was obtained on the portion of the specimen that was treated by the "Löwenstein" method and in Case L55 on the "whole blood" culture. Both of these saprophytes were slow to appear on the original plates, but on subculture they grew freely both at incubator and at room temperature. This shows the need for careful identification of any cultures that are positive on smear, especially those that are atypical on colony morphology.

Table II shows the comparative results obtained by the whole blood culture and by the Löwenstein method in the four positive cases.

TABLE II

A DETAILED STUDY OF THE FOUR POSITIVE CASES SHOWING THE COMPARATIVE RESULTS OBTAINED ON DIRECT CULTURE OF WHOLE BLOOD AND AFTER TREATMENT BY THE LÖWENSTEIN METHOD

CASE	AGE	SPECIMEN	DATE	CONDITION OF PATIENT	RESULTS OF BLOOD CULTURE				
					DIRECT PLATING—WHOLE BLOOD			LÖWENSTEIN'S METHOD	
					AMT. CULT. IN C.C.	BORDET-GENGOU	CONGO RED EGG*	AMT. CULT. IN C.C.	CONGO RED EGG*
L 11	9 mo.	1st	2/ 1/34	Pulmonary and miliary T.B.	3.5	+ 1 col.	0	3.5	0
		2nd	2/ 8/34	Patient died 2/13/34	2.5	0	0	2.5	+
L 13	9 to 11 mo.	1st	2/ 1/34	Miliary T.B.	3.5	0	0	3.5	0
		2nd	2/ 8/34		2.5	0	+ 1 col.	2.5	0
		3rd	2/15/34		2.5	0	0	2.5	0
		4th	2/22/34		4.0	0	0	4.0	+
		5th	3/ 1/34		3.5	0	0	3.5	0
		6th	3/ 8/34	Patient died 3/15/34	4.0	0	0	4.0	0
L 20	46 yr.	1st	2/ 8/34	Pulmonary T.B. critically ill	3.0	+ 2 cols.	+ 1 col.	3.0	0
L 36	13 yr.	1st	3/ 1/34	Pulmonary T.B. weak	4.0	0	0	4.0	+ mic.†
		2nd	3/20/34		5.5	0	0	5.5	0

\*Löwenstein's Congo red egg medium.

†Mic. = microscopic. No macroscopic growth was obtained after two months' incubation, but a smear of the scrapings of the culture showed a small clump of acid-fast bacilli. Subcultures of the scrapings of this culture and guinea pig inoculation gave negative results.

The results in this table show that the number of viable tubercle bacilli in the blood is extremely small and that they appear only intermittently even shortly before death. Two of the patients, L 11 and L 13, died soon after the last specimens were taken and both showed miliary tuberculosis. Yet in L 11 only one colony was obtained in the first specimen when 7 c.c. of blood was cultured and only a scant growth in 5 c.c. of the second specimen which was taken five days before death occurred. In Case L 13 only 2 of the 6 specimens gave a positive result: 1 colony from 5 c.c. of blood and a scant growth from 8 c.c. of blood. Case L 20 gave 3 colonies in 6 c.c. of blood. The first specimen of Case L 36 gave a doubtful positive culture on the portion that was treated by the Löwenstein method, although a total of 8 c.c. of blood was cultured. This culture remained macroscopically negative after two months' incubation, but a smear of the scrapings from the surface of the tubes showed some small clumps of acid-fast bacilli. Cultures and guinea pigs that were inoculated with these scrapings gave negative results. It seems likely that those were clumps of dead bacilli, otherwise they would in all probability have grown on subculture or guinea pig inoculation. A second specimen of 11 c.c. of blood gave negative results.

When the "whole blood" cultures are compared with the cultures by the Löwenstein method, we find that the results were better on the "whole blood" than on the "Löwenstein" cultures. There were 3 macroscopically positive cultures on the whole blood and only 2 on the Löwenstein culture. The culture that showed a few acid-fast bacilli in the scrapings of the Löwenstein tubes may be considered only as a doubtful positive.

There were few contaminations on the "whole blood" cultures. Although there were some contaminating colonies on the plates, none of the specimens were overgrown; whereas 7 of the "Löwenstein" specimens were overgrown by contaminations.

#### SUMMARY

A comparative study was made on 98 specimens of blood from 63 tuberculous patients and 2 specimens from a case of actinomycosis of the jaw, in order to determine whether the hemoglobin in human blood interferes with the growth of tubercle bacilli as stated by Löwenstein. The specimens were divided into 2 equal parts; one part of the blood was cultured on Bordet-Gengou and on Congo red egg mediums without any treatment, and the other part was treated according to Löwenstein's method.

It was found that the whole blood gave better results than the portion treated by the Löwenstein method; as there were 3 macroscopically positive cultures on the whole blood and only 2 on the Löwenstein cultures. In one additional Löwenstein culture a few small clumps of acid-fast bacilli were found microscopically when the surfaces of the tubes were scraped but subcultures and guinea pigs that were inoculated with these scrapings gave negative results.

Tubercle bacilli were found in only 4 of the 46 cases of advanced pulmonary tuberculosis, a large number of whom were critically ill, and 2 of the 4 patients that gave positive blood cultures died soon after the last cultures were made. All the other cases in this series gave negative results.

In two instances saprophytic acid-fast bacilli were found in the cultures. This shows the need for identifying carefully the cultures obtained, especially if they are atypical on colony morphology.

#### CONCLUSION

1. From the results obtained in this series it appears that the hemoglobin in human blood is not inhibitory to the growth of tubercle bacilli, since cultures of whole citrated blood gave better results than blood that was treated by Löwenstein's method to remove the hemoglobin.

2. Culturing whole blood has the particular advantage over Löwenstein's method of culture in that it requires no preliminary treatment and is, therefore, very much simpler and less time consuming. The use of plates instead of tubes as recommended by Löwenstein has the advantage of a large surface on which the specimen is well distributed and a slight contamination seldom overgrows the culture. We found in another investigation that when the number of tubercle bacilli is very small, growth usually takes place on the plates rather than on the tubes. Whether this is due to a better distribution of the inoculum, to a greater supply of oxygen or to other conditions, we have not been able to determine.

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# STUDIES ON INFECTIONS OF HUMAN PARASITIC WORMS UNDER INSTITUTIONAL CONDITIONS\*

WILLIAM HUGH HEADLEE, M.S.

## I. INTRODUCTION

MANY surveys have been carried out among population groups to determine the incidence of human entozoa. These surveys have included such population groups as armies, inmates of jails, hospitals, schools, orphans' homes, and asylums. The results of these surveys often give an indication of the incidence of helminth infections in a given geographic area that could not otherwise be easily obtained. This is particularly true when people are examined as they enter these institutions, the examinations of persons as they entered hospitals and jails having furnished valuable data concerning the incidence and intensity of infection and the geographic distribution of various species of human parasites.

During the past year the author carried out a survey at the Kankakee State Hospital (Illinois), adding similar data from another institution and locality.

## II. MATERIALS AND METHODS

Arrangements were made for carrying out a survey at the Kankakee State Hospital to determine the incidence of helminth infection among the patients. The population of this institution is approximately 4,000 persons, including both males and females.

In this survey three different types of diagnostic methods were used: the direct smear, the Willis flotation method, and the method of perianal scrapings. In some instances all three types of diagnostic methods were used on the same patient, others received examination by two of the methods, while a third group was examined by only one method.

*The direct smear* was made in the usual manner, with the exception that decinormal sodium hydroxide was used as a diluent. The sodium hydroxide dissolves the mucus and admits a more even film preparation, at the same time freeing the ova from fecal material to a certain degree.

*The Willis flotation method* (Willis, 1921) with slight modifications was used to make a large number of the examinations.

*Perianal scrapings* were taken from the patients in the ward by a physician assigned for that purpose. They were taken by means of a blunt instrument, a considerable area of the anal folds and perianal region being scraped. The

\*Contribution from the Zoological Laboratory of the University of Illinois, No. 468. This study was made possible by a Fellowship Grant to the author from the International Health Board of the Rockefeller Foundation.

Received for publication, October 25, 1931.

material was transferred to a slide on which a drop of decinormal sodium hydroxide had been placed. The drop of material was confined by a rectangular area which had been previously marked off on the slide by means of a wax pencil. The material was spread evenly over the area with a toothpick and immediately examined for the presence of ova, or was set aside for examination at a more convenient time. It was found that even though the preparation dried up, the ova (*Enterobius vermicularis*) were apparently not harmed enough to prevent an accurate diagnosis at a later time. When the examination was made at a later time a drop of water was added to the preparation and spread over the area so that visibility of the ova would be increased. These slides may be examined after several days or weeks and the ova will be only slightly deteriorated.

### III. PROCEDURE AND RESULTS OF THE SURVEY

The survey was carried out during the winter months of the year 1932-33. Stools were collected and examined from patients on ten different wards. Data from one other ward were obtained when perianal scrapings were made. Table I gives the information concerning the number of examinations made by these various methods and the results obtained.

A total of 876 examinations were made on stools or perianal scrapings from 652 patients. Of these 115 were males and 537 females. Infection with

TABLE I  
COMPARISON OF DIAGNOSTIC METHODS FOR *ENTEROBIUS VERMICULARIS*

WARD	NO. OF PER- SONS ON WARD	EXAMINATION BY DI- RECT SMEAR			EXAMINATION BY SALT FLOTATION (WILLIS-MOLLEY TECH- NIC)			EXAMINATION BY PERI- ANAL SCRAPING		
		NUMBER EX- AMINED	NUMBER POS- ITIVE	PER CENT POS- ITIVE	NUMBER EX- AMINED	NUMBER POS- ITIVE	PER CENT POS- ITIVE	NUMBER EX- AMINED	NUMBER POS- ITIVE	PER CENT POS- ITIVE
A-2-N	50				12	1	8.33			
A-3-N	47				13	0	0.00			
B-1-N	45				17	0	0.00			
B-2-N	44				22	0	0.00			
B-3-N	32				29	0	0.00			
1-N	206	89	0	0.00	194	12	6.19	128	45	35.16
3-N	25				19	0	0.00			
4-N	48				45	0	0.00	39	0	0.00
7-N	108				102	3	2.94			
8-N	146				52	1	1.92			
3-S	123							115	17	14.78
Total		89	0	0.00	505	17	3.37	282	62	21.99

only one species of helminth, *Enterobius vermicularis*, was noted. Seventeen or 14.8 per cent of the male patients examined were found to be infected with *Enterobius vermicularis*, while 57 or 10.6 per cent of the women were found to harbor this parasite. It must be taken into consideration when making comparisons that all men were examined by the perianal scrapings method only, while some of the women received examination by three different methods. Of all patients examined by various methods, 74 or 11.35 per cent were found

to be infected with this helminth. An adult worm was found in the stool from one of the female patients. Examinations were begun on the patients of ward 1-North and the first 89 stools collected were examined by both the direct smear method and the Willis flotation method. Examinations were continued on this ward and various women's wards by means of the Willis flotation method until stools from 505 patients had been examined. There were 3.37 per cent of these patients who were found to be infected with *Enterobius vermicularis*.

It was decided to continue examinations using a method which had been designed especially for diagnosis of infections of *E. vermicularis*, namely, the method of examination of perianal scrapings. Some of the patients were re-examined by this method and some new patients were examined. A total of 282 perianal scrapings were made and examined, including scrapings from 147 patients who had not been previously examined by another method. There were 21.99 per cent of the patients examined by this method who were found to be infected with *Enterobius vermicularis* as indicated by the presence of ova.

and taenia should be detected by this method. During the time that this survey was being carried out, the Willis flotation method was tested by means of a positive case of *Hymenolepis nana* from a source outside the institution. There was no difficulty in finding the ova in the film, even though the case was a light one.

From these discussions and the results of the author's test experiments, it would appear that other infections than *E. vermicularis* would have been detected if present, unless extremely light. It must be pointed out that these three methods are not equally efficient in detecting the presence of infections of *Enterobius vermicularis*. Table I gives us the range of this variability of incidence when different methods are used, ranging (in ward 1-North) from no infections in 89 examinations by the direct smear method to 35.16 per cent infection in 128 examinations of perianal scrapings, while the Willis flotation method would indicate that 6.19 per cent of 194 patients are infected with *E. vermicularis*.

A review of the literature of 28 surveys that were carried out on five different continents and a number of islands shows that there is a marked variability in the incidence of *Enterobius vermicularis*, as recorded. Part of this variability is no doubt due to a number of factors such as the type of population group surveyed, the age, and the environmental conditions. There is considerable evidence that much of this variability is due to the type of diagnostic method used.

Serbinow and Schuhmann (1927) call attention to the fact that the method of perianal scrapings gives more accurate diagnosis of *Enterobius vermicularis* than any of the other methods that have been used. By this method they found that as high as 87.5 per cent of certain groups of children under institutional conditions were infected with *E. vermicularis*. They showed that the total percentage of incidence is increased if more than one examination is made.

Schauchat (1931) compared technic for the diagnosis of *E. vermicularis* using Fülleborn's method, perianal scrapings, and the search for worms in the stools after an anthelmintic was administered. Thus 299 cases were examined by the Fülleborn method and 10.7 of these cases were positive; 299 perianal scrapings were examined, and 48.2 per cent were positive. After giving an anthelmintic to 196 persons, the stools were searched and *E. vermicularis* was found in 72.9 per cent.

The results of the present survey support the evidence given by Serbinow and Schulmann (1927) and Schauchat (1931), that the perianal scraping is the only method that can be used in the diagnosis of infections of *Enterobius vermicularis* with any degree of reliability.

#### V. GENERAL CONSIDERATIONS

1. *Institutional Environment and Helminth Infections.*—As has been mentioned, it is strikingly unusual that infection by only one species of helminth was noted in a group of this type of persons. So far as the author is aware, these results have not been paralleled in any other survey of population groups. Two general reasons may be set forth. One is that the technic would

probably fail to detect certain infections, but it will detect certain other infections which one might expect in an institution or similar population group. Let us then for the moment give our consideration to a second factor, namely, the effect the institutional environment has on the presence of helminth infections.

The conditions and regulations of this institution are such that there seems to be little opportunity for the spread among the patients of infections that depend upon gross pollution of surroundings to effect this transfer. The buildings are of first-class stone construction, with hardwood floors and modern plumbing and heating fixtures. Bathrooms and washrooms are supplied with water from a central water supply coming from deep wells. There are self-flushing toilets in the washrooms. Equipment, interior of buildings, and dormitories are kept scrupulously clean.

The patients are not turned out into fenced inclosures as is the case in some institutions, and there is no chance for soil pollution. Untidy or careless patients are kept in a screened or enclosed porch, depending on the season of the year. Any sputum or excreta that may reach the floor are immediately removed and the floor scrubbed with disinfectant. There is no problem of soil pollution and the ova of these forms that require an incubation period outside of the host or must develop into a larval stage would no doubt be destroyed before reinfection could occur.

Lane (1913) in reporting on the incidence of infections in prisons and jails of Bengal, regards the possibility of infection in a jail as nonexistent, and expressed an opinion that the whole question of the prevention of infection is a matter of simple hygiene such as is obligatory in a jail. He states that under such circumstances a natural cure will ensue, provided the sentences are long enough.

Keller (1931) adds some interesting data of the same import while making a study of the parasites of children in one of the state institutions just outside Nashville, Tennessee. Under proper hygienic conditions, the group of patients who did not receive treatment over a period of fifteen months showed a reduction in incidence of 100 per cent in the cases of ascaris, 83.4 per cent in the cases of *Hymenolepis nana*, 75.6 per cent in the case of trichuris, and 59 per cent in the cases of hookworm infection. The Stoll dilution egg-count method was used in these studies. Chandler (1929) in studying the rate of loss and acquisition of hookworms stated that the degree of infection in an Indian jail had lessened by 92 per cent in five years but that the number of infected persons had not.

In making a study of the helminth infections in some of the institutions of Alabama for the insane and mentally defective, Caldwell, Caldwell and Davis (1930) found that there was an increase of infection with ascaris and trichuris in incidence and degree, and that infections with hookworms increased unless modified by treatment. They, however, found that this increase was brought about by dooryard pollution and continual contact with polluted soil. The patients were permitted access to walled enclosures, and soil pollution occurred within these areas.

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1. *Institutional Environment and Helminth Infections.*—As has been mentioned, it is strikingly unusual that infection by only one species of helminth was noted in a group of this type of persons. So far as the author is aware, these results have not been paralleled in any other survey of population groups. Two general reasons may be set forth. One is that the technique would

probably fail to detect certain infections, but it will detect certain other infections which one might expect in an institution or similar population group. Let us then for the moment give our consideration to a second factor, namely, the effect the institutional environment has on the presence of helminth infections.

The conditions and regulations of this institution are such that there seems to be little opportunity for the spread among the patients of infections that depend upon gross pollution of surroundings to effect this transfer. The buildings are of first-class stone construction, with hardwood floors and modern plumbing and heating fixtures. Bathrooms and washrooms are supplied with water from a central water supply coming from deep wells. There are self-flushing toilets in the washrooms. Equipment, interior of buildings, and dormitories are kept scrupulously clean.

The patients are not turned out into fenced inclosures as is the case in some institutions, and there is no chance for soil pollution. Untidy or careless patients are kept in a screened or enclosed porch, depending on the season of the year. Any sputum or excreta that may reach the floor are immediately removed and the floor scrubbed with disinfectant. There is no problem of soil pollution and the ova of these forms that require an incubation period outside of the host or must develop into a larval stage would no doubt be destroyed before reinfection could occur.

Lane (1913) in reporting on the incidence of infections in prisons and jails of Bengal, regards the possibility of infection in a jail as nonexistent, and expressed an opinion that the whole question of the prevention of infection is a matter of simple hygiene such as is obligatory in a jail. He states that under such circumstances a natural cure will ensue, provided the sentences are long enough.

Keller (1931) adds some interesting data of the same import while making a study of the parasites of children in one of the state institutions just outside Nashville, Tennessee. Under proper hygienic conditions, the group of patients who did not receive treatment over a period of fifteen months showed a reduction in incidence of 100 per cent in the cases of ascaris, 83.4 per cent in the cases of *Hymenolepis nana*, 75.6 per cent in the case of trichuris, and 59 per cent in the cases of hookworm infection. The Stoll dilution egg-count method was used in these studies. Chandler (1929) in studying the rate of loss and acquisition of hookworms stated that the degree of infection in an Indian jail had lessened by 92 per cent in five years but that the number of infected persons had not.

In making a study of the helminth infections in some of the institutions of Alabama for the insane and mentally defective, Caldwell, Caldwell and Davis (1930) found that there was an increase of infection with ascaris and trichuris in incidence and degree, and that infections with hookworms increased unless modified by treatment. They, however, found that this increase was brought about by dooryard pollution and continual contact with polluted soil. The patients were permitted access to walled enclosures, and soil pollution occurred within these areas.



Because of the unquestionable sanitary conditions prevailing at the institution under study, and because of the possibility of losing infection with the lapse of time when the individual is not being reinfected, one would not expect to find infections with those more common worms unless in the case of late admissions.

No information is available at this institution concerning the possible increase or decrease of *E. vermicularis*, since this is the first study made. It seems probable to the author that there is a possibility of spreading the infection because of the close contact that these patients have with each other, and because of the possibility of coming in contact with clothing that is contaminated with ova.

2. *Variation of Infection with E. Vermicularis on the Different Wards.*—By observing the table containing the results of these examinations one will notice that wards 1-North and 3-South have the highest percentage of infected patients. As the patients are admitted to the hospital they are taken to the receiving wards and left there until they can be classified. Personal habits, behavior, and physical condition are criteria used in classifying patients. There is considerable shifting of patients as their condition changes. The patients on wards 1-North and 3-South are classed as untidy. Mentally they are very much deteriorated, and they are very untidy in personal habits. Although the patients on ward 4-North were examined by both the Willis technique and by perianal scrapings, no infections were found. The author was impressed by the general good appearance of the patients on this ward and could note an interest in personal hygiene. The majority of the patients on this ward were classified as having some form of epilepsy or epileptic psychosis. This seems to bear out what would be expected, that the mental condition as it affects their personal habits influences the incidence of infection.

3. *Relation of Mental State to Parasitic Infection.*—When the patients are grouped according to the type of psychosis and compared with the incidence of infection, there seems to be little indication of a correlation. The data will bear a more thorough study from this standpoint. Marehand (1923) expressed an opinion that there might be a relation between convulsions due to helminth infections and epilepsy. However, in this survey no infections were found among the epileptic patients on ward 4-North. As stated above, it would appear that infections are influenced by the mental state of the persons and their habits as brought about or conditioned by the type of psychosis.

4. *Correlation of Age and the Incidence of Infection.*—In Table II information is given concerning the incidence of infection in relation to age. When considering the wards separately, with the exception of one or two, the number of patients in each age group is so small that a comparison cannot be made with any degree of accuracy. Considering all of the patients as a group there is no marked correlation of infection with the various age groups. The incidence in the age groups from twenty to twenty-nine and from thirty to thirty-nine seems to be uniformly lower than the age groups above them, but not markedly so. There were only three persons under twenty years of age and none of these were infected.

TABLE II  
CORRELATION OF AGE AND INCIDENCE OF INFECTIONS

AGE GROUP	TOTAL INDIVIDUALS										INFECTED INDIVIDUALS ( <i>E. vermicularis</i> )										PER CENT INFECTED										TOTALS						
	A-2-N	A-3-N	B-1-N	B-2-N	B-3-N	1-N	3-N	4-N	7-N	8-N	3-S	A-2-N	A-3-N	B-1-N	B-2-N	B-3-N	1-N	3-N	4-N	7-N	8-N	3-S	A-2-N	A-3-N	B-1-N	B-2-N	B-3-N	1-N	3-N	4-N	7-N	8-N	3-S	PERSONS	PER CENT INFECTED	PER CENT INFECTED	
15-19						2					0											0												3	0	0.00	
20-29						2	3	5	20	2	5	15	4	29								0												87	8	9.19	
30-39	7	8	6	8	9	62	7	11	37	9	27	0	0	0	0	13	0	0	2	0	2		0	0	0	0	0	0	20.9	0	5.4	0	7.4	191	17	8.90	
40-49	3	3	4	3	7	63	5	16	37	18	29	1	0	0	0	18	0	0	1	0	5		33.3	0	0	0	0	0	29.0	0	0	2.7	0	17.2	188	25	13.29
50-59	2	3	7	5	46	2	8	11	15	16	0	0	0	0	11	0	0	0	1	3	0		0	0	0	0	0	23.9	0	0	0	6.7	18.8	115	15	13.04	
60-			2	3	30	3	8	2	6	13						0	0	0	0	0	2						0	23.3	0	0	0	33.3	67	9	13.43		
Age not stated			1													0												0						1	0	0.00	

## VI. SUMMARY

1. A survey was carried out at the Kankakee State Hospital to determine the incidence of helminth infections among the patients.

2. Methods of diagnosis used were the direct smear, the Willis flotation method, and the perianal serapings method. Some patients were examined by all three methods. Slide preparations of perianal serapings set aside for several hours or days before diagnosis gave accurate diagnosis of *Enterobius vermicularis*.

3. Of 652 patients 876 examinations were made. Only ova of *E. vermicularis* were found, being present in 74 or 11.35 per cent of all of the patients examined. Some wards were negative: in other groups 35 per cent of the individuals examined were found infected with this helminth.

4. An evaluation is given of the diagnostic methods used. Results indicate that the examination of perianal serapings is the only method that approaches reliability in the diagnosis of *Enterobius vermicularis* infection. When using this method, examinations should be made at several different times before a final negative diagnosis is given.

5. The institutional environment is such that little opportunity is afforded for the spread of this infection among patients if it depends on gross pollution of surroundings. *Enterobius* may possibly be spread from person to person through contact with soiled clothing, or because of intimate association. Other infections that may be present when the patient is admitted, such as ascaris and trichocephalus, will likely be lost without further spread.

6. Infection with *E. vermicularis* varies in the different wards. The wards having a high percentage of mentally deteriorated individuals have a higher incidence of infection.

7. There is little correlation between the type of psychosis and the infection other than that the psychosis influences the habits of the individual, in some cases predisposing to helminth infection.

8. There is no marked variation in the incidence of infection with *E. vermicularis* among the different age groups.

The author wishes to express his appreciation to Dr. Henry B. Ward for his interest and encouragement in carrying out this survey and for the many helpful suggestions received. Thanks are also due Dr. G. W. Morrow, Acting Managing Officer of the Kankakee State Hospital (Illinois), for placing before me the facilities of the hospital which made it possible to carry out this survey.

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## THE INFLUENCE OF CERTAIN LIPOIDS ON THE GROWTH OF A RABBIT NEOPLASM\*

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ALVIN R. HARNES, M.D., NEW YORK, N. Y.

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EARLY in the study of a malignant tumor, isolated from the rabbit by Brown and Pearce,<sup>1</sup> two peculiarities were noted. First, metastasis of the tumor occurred rarely in the brain or central nervous system of the animal. Second, the tumor would rarely grow from a primary inoculation in the brain of the rabbit. Later studies by Harnes<sup>2</sup> showed that animals with a high whole blood cholesterol recovered from the tumor, while those animals with low whole blood cholesterol died. It was also pointed out in this study<sup>2</sup> that as the tumor progressed in growth and metastasis, the whole blood cholesterol determinations increased in value.

With these facts in mind it was thought desirable to study the effect of certain lipid extracts of brain tissue on the growth and metastasis of this rabbit neoplasm.

### MATERIALS AND METHODS

The cerebrum, cerebellum, and medulla of calves, aged from six to eight months, were obtained and the extract prepared within twenty-four hours after the animal was killed. After removing the meninges, the brain tissue was finely minced and placed in a liter flask. Three hundred cubic centimeters of neutral 95 per cent  $C_2H_5OH$  were then added and the whole refluxed under vacuum at 70° C. for one hour. The alcohol was then drained off and filtered. After removing the alcohol by distillation the residue was taken up with corn oil in sufficient amount to make a 50 per cent solution of brain extract. This solution will hereafter be spoken of as brain extract.

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\*From the Department of Pediatrics, Cornell University Medical College and the New York Hospital.  
Received for publication, November 16, 1934.

The rabbits used in this experiment were of mixed breeds and from six to eight months of age. They were kept in the same room and given the same diet. The 33 animals were divided into three groups: 11 for controls, 10 for corn oil, and 12 for brain extract. Prior to the time of inoculation with the tumor, the latter group received 5 c.c. of brain extract subcutaneously on alternate days until a total of 30 c.c. had been given. The other received 2.5 c.c. corn oil in like manner on the same days. On April 15, 1933, all animals were inoculated in one testicle with 0.3 c.c. of a tumor emulsion. The animals described above continued to receive brain extract and corn oil until the termination of the experiment on June 12, 1933. On this date all animals were killed and autopsied.

#### KIDNEYS CONTAINING TUMOR METASTASES IN PER CENT CONTROL

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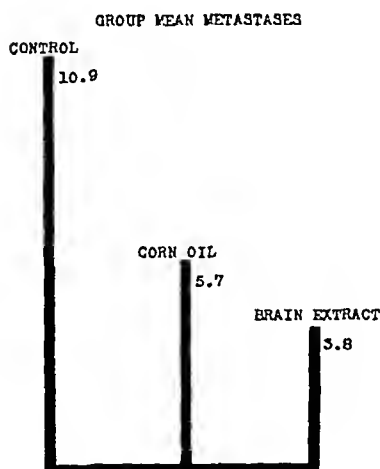


Fig. 1.

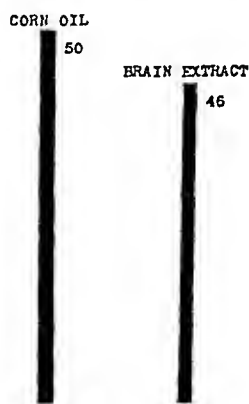


Fig. 2.

Fig. 1.—Group mean metastases.

Fig. 2.—Kidneys containing tumor metastases in percentage.

#### RESULTS

The results of the observations made in this experiment are presented in the form of tabulated summaries, Table I, and Figs. 1 and 2. The tabulation represents the number of metastases of the neoplasm per animal and includes the primary tumor.

#### DISCUSSION AND CONCLUSIONS

From the results presented in Table I it will be noted that the highest number of metastases occurred in the control rabbits. The mean value for the eleven animals in this group was  $10.9 \pm 0.71$  with a standard deviation of 3.5. The number of kidneys free of tumor in this series was found to be 29 per cent (Fig. 2). The volume or amount of tumor was greatest in these animals.

Those animals receiving corn oil had the best appearance clinically. They maintained a constant weight and were entirely free of snuffles at the termina-

TABLE I  
NUMBER OF METASTASES PER ANIMAL

11 CONTROLS	10 CORN OIL	12 BRAIN EXTRACT
8	14	2
15	8	4
12	2	8
16	2	1
12	5	7
12	6	2
9	5	4
4	6	6
15	6	5
8	3	3
9		1
		3
Mean = $10.9 \pm 0.71$	Mean = $5.7 \pm 0.73$	Mean = $3.8 \pm 0.48$
St. D. = 3.5	St. D. = 3.25	St. D. = 2.34

tion of the experiment. The mean value for the ten animals in this series was  $5.7 \pm 0.73$  (Fig. 1) with a standard deviation of 3.25. Fifty per cent of the kidneys were free of metastatic tumor.

The twelve animals receiving the brain extract had the least amount of tumor and number of metastases. Clinically these animals were in very poor condition, having lost considerable weight, and many were developing snuffles. The mean value for metastatic tumor in this group was  $3.8 \pm 0.48$  with a standard deviation of 2.34. Fifty-four per cent of the kidneys were found free of neoplasm.

#### SUMMARY

The results of an experiment are presented in which it is shown that an alcoholic extract of brain and corn oil retard the growth and metastasis of a malignant rabbit neoplasm. The average number of metastases for the control animals was found to be  $10.9 \pm 0.71$ ; for those animals receiving corn oil  $5.7 \pm 0.73$ ; and those receiving brain extract  $3.8 \pm 0.48$ . The standard deviation for these groups was 3.5, 3.25, and 2.34, respectively.

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# LABORATORY METHODS

## AN EXPERIMENTAL BLOOD SAMPLING PROCEDURE\*

GEORGE S. SAMUELSEN, B.S., BROOKLYN, N. Y.

**M**ETABOLIC studies involving blood chemistry procedures upon rabbits have been greatly hampered by the difficulty encountered in obtaining the necessary blood samples. It was an attempt to remedy this condition that resulted in the development of the following procedure. This method is a simplification of one in common use, but has the added advantage of being rapid, of being performed without the aid of an assistant, and apparently causing but slight damage to the animal.

*Procedure.*—The rabbit is allowed to sit unrestrained upon a table. The operator's hands are then placed one on each side of the animal (Fig. 1), in



Fig. 1.

such a manner that the location of the heart is clearly defined. Now let the index finger of the left hand indicate the point of maximum beat; this is usually the fourth interspace. A 23 gauge needle having a one-inch shaft, attached to a convenient size syringe, is introduced into the chest wall of the animal at the indicated point, in such fashion as to keep the needle and syringe always in a horizontal plane. The heart will usually make its location known by the intermittent beating against the needle point. Upon introducing the needle still further, the wall of the heart is pierced. A slight withdrawal of the plunger will cause blood to be drawn into the syringe (Fig. 2). In many instances it is unnecessary to withdraw the plunger for the pressure within the heart will cause an unrestrained plunger to be forced slowly out of the syringe

\*Received for publication, December 31, 1934.

by the inflow of blood. When the desired amount of blood has been obtained, the needle is withdrawn in the same horizontal plane as introduced.

*Comment.*—In order to test the value of this procedure, about fifty rabbits were subjected to the treatment outlined above, as often as ten times in one day and fifty to sixty times within a six-week period. There was not the slightest sign of injury to even a single animal of the entire series. This, combined with the picture resulting from autopsy of many animals, shows this method to be practical and worthy of recording.

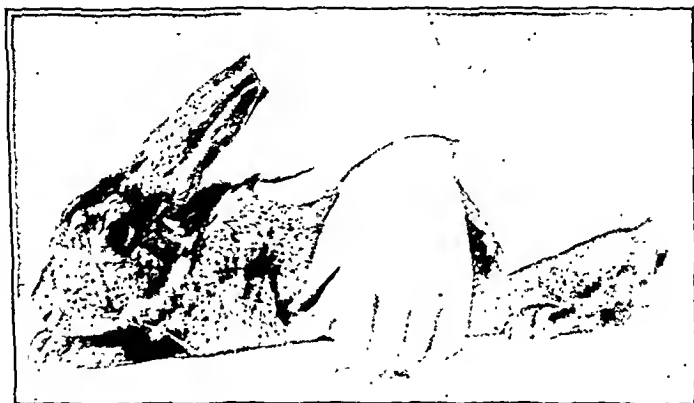


Fig. 2.

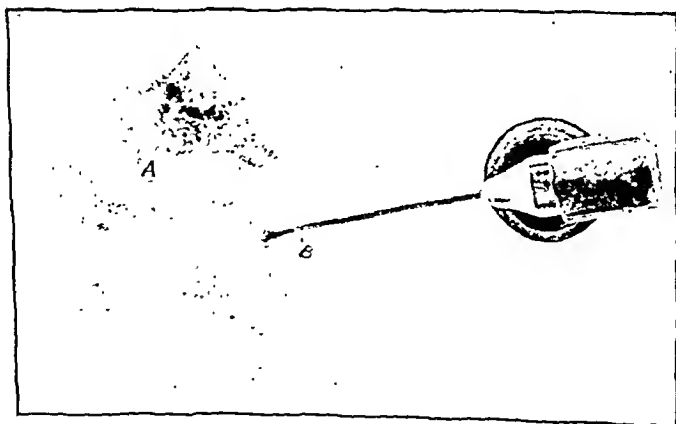


Fig. 3.

In many cases, the needle was introduced into the heart as described, and the animal killed in order fully to describe the point of penetration. In all cases there was no indication of hemorrhage. The points of introduction of the needle were completely closed. In all cases the needle pierced the left ventricle at a point on the base of the heart between the interventricular line (A, Fig. 3), and the circumflex branch of the left coronary artery (B, Fig. 3). Dissection of the heart showed that the needle entered the left ventricle just above the papillary muscle, but below the mitral valve.



The success of this procedure lies primarily in the following facts: (1) The rabbit being in the sitting position presupposes a normal and almost constant location of the heart. (2) The introduction of the needle from the left side of the animal ensures the needle striking the left ventricle. (3) Besides striking the heart at the point where the muscle wall is thickest the ventricle itself at this point has the greatest depth, thus allowing the maximum variation extent to which the needle may penetrate, without piercing the interventricular septum. This control of the extent to which the needle is introduced is further checked by using a needle of standard length.

The author takes this opportunity of expressing his sincere appreciation to Drs. Matthew Steel and G. B. L. Smith for their kind interest and suggestions.

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## ON A NEW MODEL OF PIPETTE FOR THE ERYTHROCYTE SEDIMENTATION TEST\*

GILBERTO G. VILLELA, RIO DE JANEIRO, BRASIL, S. A.

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**I**N THEIR research work Faracus and numerous other investigators have shown the value of the relative erythrocyte sedimentation rate in connection with infectious diseases, especially in tuberculosis and leprosy. Since then many different types of instruments have been brought out for measuring the speed of sedimentation.

Linzenmeier adopted as a measure, the time which the erythrocyte column takes to sink as far as 18 mm. in a tube of special width. This technique did not prove practical, especially in cases where the sedimentation was very slow, thus causing considerable loss of time.

The pipette recommended by Westergren is more accurate, and with it readings can be taken every hour, thus making it possible to plot the curve of the drop of the blood corpuscle volume during the time of the procedure.

Westergren's first model consisted of a glass tube of even thickness, to which a ruler, scaled in millimeters, was fixed and through which the corpuscular column, inside, could be read. Later, more nearly perfect tubes for direct reading were made, having the graduations marked on the glass itself. The greatest drawback of these models was the difficulty in keeping the blood column from leaking through the lower end of the tube. The hematocrit tube is always accompanied with a wooden support or pedestal to which a metal plate is fixed, for the double purpose of keeping the tube in an upright position and preventing the blood from escaping. But even with very careful handling, it was difficult to ascertain the level the blood had reached, and at the same time prevent its leaking out at the lower end of the tube.

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\*From the Oswaldo Cruz Institut.  
Received for publication, December 10, 1934.

Adler recently tried to correct these deficiencies by fitting a piece of rubber, shaped like the finger of a glove, on the upper part, which also acted to retain the blood, by suction.

The Adler pipette widened out at the upper end and was fastened to a rest or prop by means of two side hooks which compressed the rubber band, to stop the blood from escaping.

In this description, we are proposing a new and simplified model, which is easily handled and which appears to us to overcome, and do away with all the defects of former types.

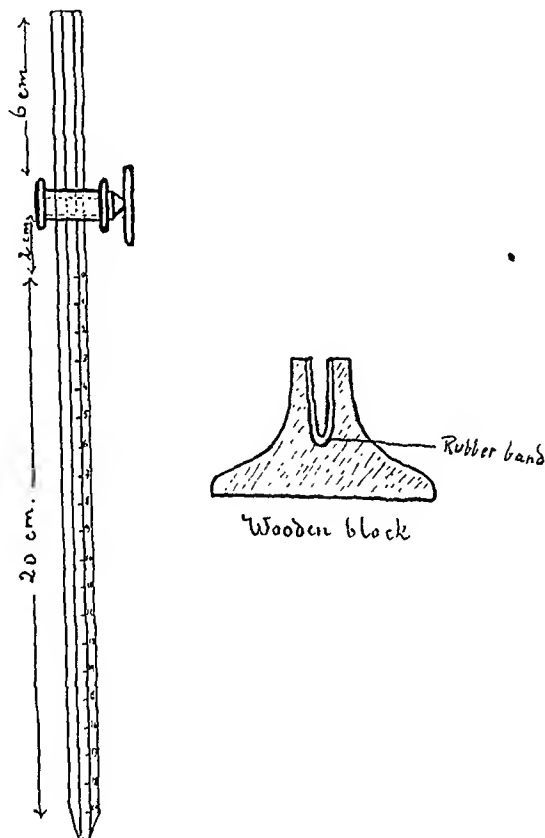


Fig. 1.—Sedimentation pipette.

Our sedimentation tester is composed of a tube similar to Westergren's, that is, it is the same in width and also sealed to millimeters, but it has a tap attached to the upper end, which allows perfect control over the levelling of the contents of the tube, so that both Westergren's pincers as well as Adler's fittings of rubber may be dispensed with (see Fig. 1). Besides this it may be noted how easily the pipette can be operated. It can be emptied at will and regulated by a mere turn of the tap.<sup>1</sup> The pipette is kept upright in a little wooden stand with a small hole in it, which adapts itself perfectly, with the aid of a circular rubber band on the edge of the rim in which the tube is placed.

<sup>1</sup>The tap must be lubricated with lanoline or other kind of grease to avoid the loss of air.

The readings can be taken exactly as with Westergren's apparatus, and the results may be compared because the diameter and the sealing of the tube are identical.

The technic for the determination is simple:

Place 0.1 c.c. of a 15 per cent solution of neutral potassium oxalate in a test tube, and then dry it carefully by holding it over a flame. Draw, by venipuncture, 5 c.c. of blood and introduce it immediately into the tube and shake it slightly. Draw blood up in the pipette to a little above the sign *O*, and turn the tap off. Fix the level with a little movement of the tap and wipe away the blood from the outside wall of the tip of the tube with a piece of filter paper. A concave méniscus forming at the lower end of the pipette, will show that the shutting off by the tap was absolutely successful.

Now place the pipette upright on the opening of the wooden block and make readings at one, two, six and twenty-four hours from the time the blood was drawn.

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## A NEW DEVICE FOR OXYGEN ABSORPTION IN GAS ANALYSIS APPARATUS\*

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F. S. COTTON, D. SC., BOSTON, MASS.

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IT IS doubtless the experience of all workers in the field of gaseous metabolism that potassium pyrogallate solutions are frequently found to be unduly slow in absorbing oxygen, so necessitating the tedium of many trips to the absorption pipette.†

Further, the adoption of the Krogh type of absorption pipette where greater reliability of analysis is in question, accentuates still more this tedium. The Krogh pipette (series of pear-shaped guttered bulbs) is shown in Fig. 1, *A*. This pipette eliminates the risk of entangling small air bubbles by increasing liquid surface without resorting to the original series of hollow internal tubes, but in abandoning the latter, sacrifices an extensive amount of useful surface, and so lengthens (relatively) the absorption process.

This drawback to the Krogh improvement has stimulated the production of various devices to obviate the trouble. All of these involve at least sufficient complexity to increase distinctly the expense of manufacture, while the presence of moveable parts in some involves in addition the possibility of clogging with use, or of occasionally entangling a small gas bubble. Nothing but very extensive use could yield a pronouncement on the latter points. Shepherd, Martini, and Margaria have each described devices for the purpose in question.

The justification for yet another device is that it is simple of construction, works automatically, and having no moveable parts, cannot become clogged or entangle air bubbles.

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\*From the Fatigue Laboratory, Morgan Hall, Harvard University.

Received for publication, January 16, 1935.

†There is nothing in the literature to account for this irregular behavior, and research instituted along the lines of catalysis with the object of hastening the process has so far been fruitless.

The objects of the present design are:

1. To extend the stay in the pyrogallate of the last half of the air which visits it, which half is, of course, normally the first to return.
2. To promote the amount of contact between gas and fluid surfaces in the pipette.
3. To keep fresh pyrogallate flowing over the glass surfaces, which typically drain only too readily.\*

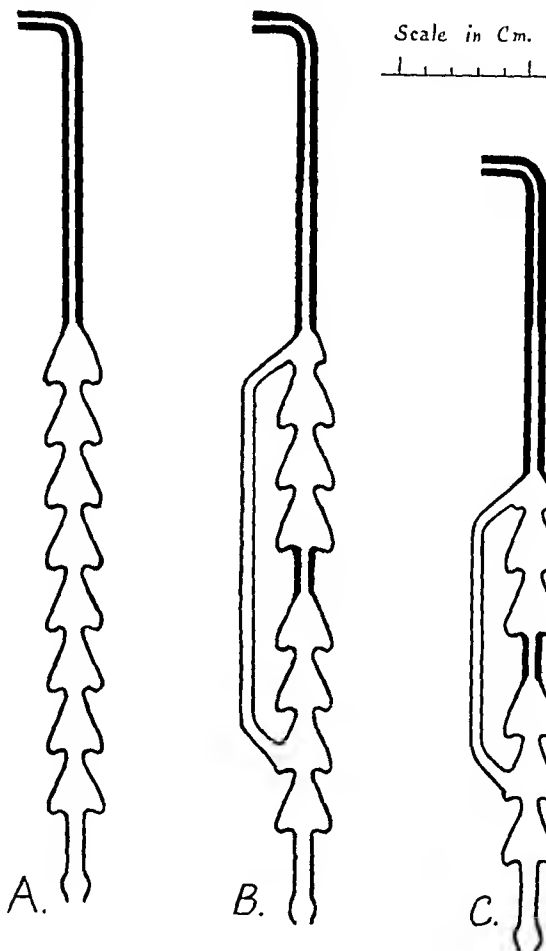


Fig. 1.—A, Krogh pipette. B, modified device for 20 c.c. gas machine. C, modified device for 10 c.c. gas machine.

The way in which these objects are attained may be illustrated by reference to Fig. 1.

The first object is secured as follows: As the gas is forced over into the pipette (Fig. 1, B), the pyrogallate is driven more quickly through the side tube than through the series of bulbs, since the constriction shown offers more resistance to flow. Approximately when the first half portion of gas has entered the pipette, it has begun to pass through the lower end of the side tube, so that most of the second half portion is thus manipulated below the first and, therefore, retreats after it when the gas is withdrawn from the pipette.

\*Hence the gutters in the Krogh bulbs which are designed to restrict too radical a drainage. Further, the mechanical pump of the 40 c.c. Carpenter machine for chamber air analysis is a practical proof of the value of continuous irrigation.

The second object is secured in two ways: (a) When the second half portion of gas enters the main body of the pipette by the lower end of the side tube, the gas bubbles up through the fluid, so securing optimal physical contact. Moreover as soon as a cubic centimeter or so collects there, it is sprayed from above by the descending jet of pyrogallate traveling fast through constriction. (b) When the withdrawal of gas commences, the pyrogallate is very quickly drawn back up the side tube, and, spilling over into the gutter of the top bulb descends as a curtain of fluid all round as the air retreats, thus bringing fresh fluid into contact with this air.

It remains to quote precise facts about the relative efficiency of this device in comparison with the original Krogh system of bulbs.

This comparison was made by using the same solution of potassium pyrogallate to absorb the oxygen from samples of air in each of the pipettes in ques-

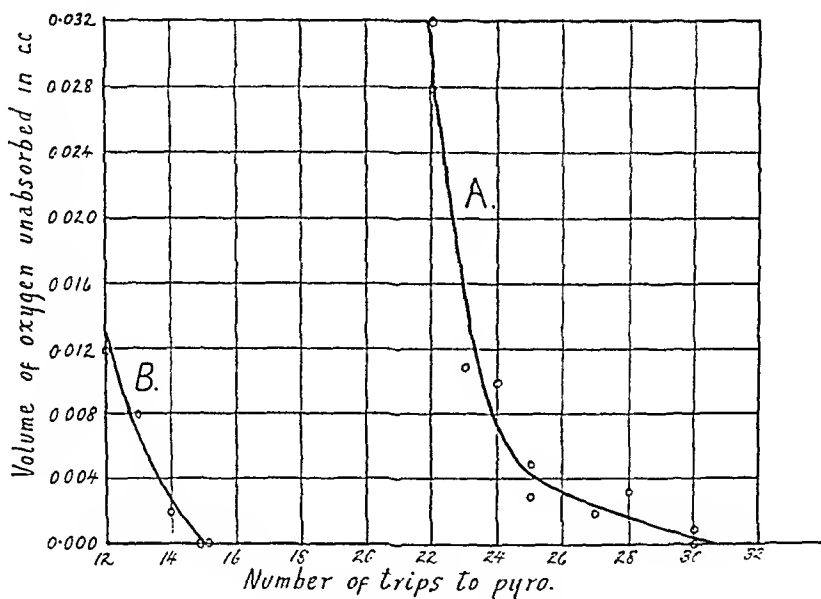


Fig. 2.—A, Krogh pipette. B, Present device.

tion. The number of trips to pyrogallate requisite to absorb completely the oxygen was ascertained in the following manner. In the case of each sample of air, of approximately the same initial volume, a certain number of trips ( $n_1$ ) were made to the pyrogallate and the resultant volume observed. A further number of trips was then made in excess of the number required for complete absorption of the oxygen, and this resulting volume also was recorded. On repeating this procedure a number of times, sufficient data were obtained to plot the residual volume of oxygen (difference of the reduced readings on the buret) against the initial number of trips to pyrogallate ( $n_1$ ).

Fig. 2 shows the result in the case of a Krogh pipette for absorbing oxygen from a 20 c.c. sample of air, and in the case of the present device.

The graph shows that whereas with the unmodified Krogh bulbs the necessary number of trips to pyrogallate was more than thirty, the number required

in the case of the present device was fifteen. Hence, this device cuts down the number of trips to approximately one-half. The same procedure was also adopted in comparing the Krogh pipette of a 10 c.c. Haldane apparatus with the device in question of appropriately reduced size. In this case the increased efficiency is not so great as when larger volumes are concerned, the number of trips to pyrogallate being reduced to three-quarters in comparison with the reduction to one-half in the first case.

The requisite diameters of side arm and constriction had, of course, to be determined by trial, so that the optimal result was obtained only after a number of experimental pipettes\* had been constructed, in which these measurements were suitably varied.

The dimensions found suitable for the pipettes of the two sizes discussed in the text are given below, the diameter in each case being the internal diameter. The same dimensions apply to both pipettes which differ in the number of bulbs and position of the constriction.

*Bulb dimensions—*

Neck of bulb	diameter	4.0 to 5.0 mm.
Maximum width	diameter	19.0 to 20.0 mm.
Height of bulb		15.0 to 16.0 mm.
Side tube		2.9 to 3.1 mm.
Constriction	diameter	2.3 to 2.4 mm.
	length	14.0 to 16.0 mm.
Capillary tube above bulb—		
Leading from rest of apparatus	diameter	1.8 to 2.0 mm.
Short length above top bulb	diameter	2.6 to 2.8 mm.

The longer linear dimensions are deducible from the attached scale of 5 cm.

#### SUMMARY

A device is described for use in gas analysis machines to render more efficient the contact of gas and fluid in absorption pipettes, and so to reduce the number of trips necessary for complete absorption of the gas analyzed.

By this means the number of trips may be reduced to one-half or three-quarters that which is normally necessary for the type of pipette in current use for accurate quantitative absorption of oxygen by potassium pyrogallate, the extent of the saving being greater where larger volumes of gas are concerned.

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\*My thanks are due to Arthur H. Thomas Company of Philadelphia for their generosity in supplying these experimental pipettes.

# A GENERALLY APPLICABLE METHOD FOR THE ENUMERATION OF MICROSCOPIC OBJECTS\*

A. C. FAY, PH.D., MANHATTAN, KAN.

IN THE various fields of scientific investigation it is frequently desirable to enumerate microscopic objects such as bacteria, yeasts, mold spores, blood cells, intestinal parasites or their ova, protozoa, etc. Some of these forms may best be seen with the low power, others with the high dry or the oil immersion objectives of the microscope. Fay† has suggested a method using the oil immersion objective for the enumeration of bacteria, the general principles of which may easily be adapted for general use with the other objectives of the microscope. The method is as follows:

Place 0.1 ml., or 0.1 gm., of the specimen to be examined on a chemically clean 3 by 1 inch glass slide. Quantitatively diluted specimens may be used. If necessary to facilitate spreading, a few drops of water, stain or other liquid may be added to the slide; this need not be included in the calculation of quantitative dilution of the original specimen. After spreading the specimen evenly over the entire area of the slide, the preparation may be dried, fixed and stained by a suitable process or examined in a wet unstained condition, depending upon the nature of the material. The size and number of the objects to be counted will determine which objective of the microscope should be employed.

With the aid of a stage micrometer, adjust and record the tube length of the microscope which will give diameters of 1.57, 0.351, and 0.157 mm. for the fields of the low power, high dry, and oil immersion objectives, respectively. The areas of the respective fields will be 0.003, 0.00015, and 0.00003 square inches which represent 1/1,000, 1/20,000, and 1/100,000 of the area of the slide. Obviously if 0.1 ml. of the specimen is employed, the average number of objects counted per field should be multiplied by 10,000, 200,000, or 1,000,000 as indicated in Table I to give the count per milliliter. If more or less than 0.1 ml. of sample is used, suitable adjustment of the factor can be made. In order to insure a fair count, representative fields at widely separated parts of the slide should be selected at random.

In certain types of work it is more convenient to count the total number of objects in a continuous strip across the short dimension of the slide. In this case if the diameters of the fields for the three objectives are adjusted to 1.52, 0.381, and 0.152 mm., respectively, the areas of the corresponding strips represent 1/50, 1/200 and 1/500 of the entire area of the slide. As indicated in Table II, when 0.1 ml. of sample is employed, the total number of objects counted

\*From the Kansas Agricultural Experiment Station.

Received for publication, January 31, 1935.

Contribution No. 161, Department of Bacteriology.

†Fay, A. C.: J. Dairy Sc. 16: 311, 1933.

in one strip should be multiplied by 500, 2,000, or 5,000 to give the number per milliliter. This method obviates the necessity of keeping a record of the number of fields observed, but it should be employed only when the slide is moved by means of a mechanical stage. The diameters suggested in Tables I and II usually can be obtained with a 10X ocular.

It is frequently advantageous to insert into the eyepiece an ocular disc marked into quadrants and with one circle slightly smaller than, and concentric with, the microscopic field. If the ocular disc is used, the diameter of the concentric circle rather than of the whole field is adjusted as indicated in Tables

TABLE I

FACTORS FOR CONVERTING THE AVERAGE NUMBER OF OBJECTS PER FIELD INTO THE COUNT PER MILLILITER WHEN 0.1 ML. SAMPLE IS EMPLOYED

OBJECTIVE	DIAMETER OF FIELD IN MILLIMETERS	FACTOR
Low power (16 mm.)	1.570	10,000
High dry (4 mm.)	0.351	200,000
Oil immersion (1.9 mm.)	0.157	1,000,000

TABLE II

FACTORS FOR CONVERTING THE TOTAL NUMBER OF OBJECTS IN A STRIP ACROSS THE SHORT DIMENSION OF A SLIDE INTO THE COUNT PER MILLILITER WHEN 0.1 ML. SAMPLE IS EMPLOYED

OBJECTIVE	DIAMETER OF FIELD IN MILLIMETERS	FACTOR
Low power (16 mm.)	1.524	500
High dry (4 mm.)	0.381	2,000
Oil immersion (1.9 mm.)	0.152	5,000

I and II. A 6.4X ocular is most suitable for this purpose. The use of the ocular disc restricts the vision to the center of the field and eliminates from consideration the periphery where definition is frequently indistinct.

The method is being presented because it is believed that the fundamental principles involved and the values for standardization of the microscope may be useful in a number of fields of scientific work. Details of the procedure for the preparation of slides, the choice of volumetric or gravimetric samples, the extent of dilution, the staining, etc., which would make this method of enumeration directly applicable to the work of the bacteriologist, parasitologist, mycologist, pathologist, and others necessarily will be different for each specific use.



# THE DETERMINATION OF LIPID PHOSPHORUS\*

## ALCOHOL ETHER SOLUBLE PHOSPHORUS

FRANCES KRASNOW, PH.D., A. S. ROSEN, D. PHAR., AND Y. POROSOWSKA,  
NEW YORK, N. Y.

**L**ONG-CONTINUED use of the method of Krasnow and Rosen<sup>1</sup> for the determination of lipid phosphorus† has resulted in the introduction into the original process of certain modifications. These are embodied in the present communication.

It has been found advisable to discard the gas-heated oven, owing to the practical impossibility of maintaining a sufficiently constant temperature with the gas as supplied in certain localities. The condition was corrected by the use of an electric oven, in which it was found that a temperature range of approximately 80 to 90° gives very satisfactory results, provided that the acid fumes be allowed to escape at such a rate that a considerable proportion of them is always present. This is accomplished in a 15 by 16 by 12 inch oven especially constructed to withstand the acid fumes.‡ With this equipment, ten samples (but no more) may be oxidized at one time; the reaction is complete in sixteen to eighteen hours. The effect of the above change is illustrated by figures shown in Table I, showing the number of milligrams of lipid phosphorus and *lecithin*§ calculated from the phosphorus value recovered from 100 mg. of commercial lecithin and 100 c.c. of whole blood respectively.

TABLE I

SAMPLE USED	GAS OVEN		ELECTRIC OVEN	
	P	Lecithin	P	Lecithin
1 c.c. of commercial Lecithin solution (0.1% in alcohol)	2.27	56.7	2.84	71.0
	2.27	56.7	2.86	71.5
	2.24	56.0	2.82	70.5
0.5 c.c. of whole blood	9.50	238.0	10.48	262.0
	8.56	214.0	9.00	225.0
	8.00	200.0	10.28	257.0

Since some of the measurements in the original technic have been changed, the details are here supplied:

Blood (0.5 c.c.) is pipetted into 10 c.c. of an alcohol-ether mixture (3:1) contained in a 50 c.c. volumetric flask, preferably fitted with a glass stopper, and

\*From the School for Dental Hygiene, Guggenheim Dental Clinic, and the Department of Dermatology and Syphilology at the New York Post-Graduate School and Hospital.

Received for publication, January 3, 1935.

†"Lipid phosphorus" is used throughout this paper to indicate the alcohol ether soluble phosphorus. The amounts of other phosphorus compounds which might appear in the alcohol ether extracts of the fluids studied have been stated to be negligible.

‡The construction of this oven was made possible through the cooperation of the Electric Heat-Control Apparatus Company, Newark, New Jersey.

§Although commercial lecithin is not pure lecithin, it served as a good working material since we used it for comparative purposes only.

brought to boiling by immersion in a water-bath. Digestion is continued for about three minutes. After cooling, alcohol-ether is added to the 50 c.c. mark and the whole shaken vigorously. The filtrate (25 c.c.) is transferred quantitatively to a casserole of 150 c.c. capacity and evaporated to dryness over a water-bath. Then 10 c.c. of  $\text{HNO}_3$  (sp. gr. 1.42) is added and the dish rotated in such a way that the acid loosens the dried extract from the sides. One-tenth cubic centimeter of  $\text{H}_2\text{SO}_4$  (sp. gr. 1.84), 5 c.c. of  $\text{KClO}_3$  (saturated solution) and 4 c.c. of a mixture of saturated  $\text{KNO}_3$  and a saturated solution of  $\text{NaNO}_3$  (1:1) are introduced. The casserole is placed in the oven at 80 or 90° C. and digestion continued until the mixture becomes white. A safe interval is eighteen hours. The white crystalline residue is dissolved in water by heating over a small free flame and transferred to a centrifuge tube (15 c.c.). Including at least two washings, the total volume should not exceed 10 c.c.

Following the Tisdall method<sup>2</sup> the phosphate is precipitated with 1 c.c. of the strychnine-molybdate reagent and agitated three times during fifteen minutes. After being centrifuged for three minutes at high speed, 1,600 r.p.m., the supernatant fluid is decanted and the precipitate washed twice with water, each time centrifuging at least one minute, 1.0 c.c. of  $\text{NaOH}$  (1 per cent) is added and stirred until solution is complete. This is diluted with water to about 5.0 c.c. and transferred to a 50 c.c. volumetric flask fitted with a glass stopper. The centrifuge tube is washed twice with water, using 5.0 c.c. each time, and the washings added. Ten c.c. of  $\text{K}_4\text{FeCN}_6$  solution (20 per cent) and 5.0 c.c. of  $\text{HCl}$  (sp. gr. 1.18) are introduced. The mixture is allowed to stand for ten to twelve minutes and made up to volume with water. Readings are made immediately against a phosphate standard, prepared as directed by Tisdall. The formula for calculating the lipid phosphorus, P, in 100 c.c. of whole blood is:  $P = 20/R \times 10$  where 20 is the reading of the standard and R is the reading of the unknown. To obtain the corresponding "lecithin" content, the value of P is multiplied by 25.

Tests have shown that the blood may be kept overnight in the ice chest and the procedure outlined above may be conveniently interrupted at any point except after the addition of the concentrated  $\text{HCl}$ .

One of our previous reports on lipid phosphorus contended that "test tube" oxidations should be discarded.<sup>3-10</sup> However, it was felt that with the technique of Zahnd and Clarke for manipulating such oxidations in the determination of sulphur,<sup>11</sup> reproducible results might now be obtained. As in the oven method 1 c.c. samples of a solution of commercial lecithin (0.1 per cent in alcohol), were used. The average recovery for four series of estimations were 2.64, 2.92, 2.68, 2.80 mg. P per 100 c.c., respectively, with a final average of 2.76 mg. which compares well with 2.84 mg. The steps in the procedure after oxidation were those adopted for the oven method previously described.

These data become more significant when we compare them with figures obtained on the same solution by other methods recently recommended for lipid phosphorus.<sup>12, 13</sup> The respective recoveries are 1.96 and 0.88 mg. P. However, when, in the latter technique, all the steps referring to extraction were omitted, 2.85 mg. was recovered.

The comparison is presented in Table II.

Some of these methods were then compared as to their efficiency in the determination of lipid phosphorus in whole blood. Here, too, the results may be best presented in tabular form (Table III).

TABLE II  
MG. LIPID PHOSPHORUS RECOVERED BY EMPLOYING DIFFERENT METHODS

	BENEDICT- THEIS	BLOOR	ZAHND- CLARKE	KRASNOW- ROSEN	PREGL <sup>14</sup>
	2.07	0.86	2.64	2.84	2.90
	1.83	0.86	2.92	2.86	2.90
			2.67	2.82	
			2.81	2.90	
Average	1.95	0.86	2.76	2.86	2.90
Remarks	Determination from another laboratory yielded 1.55	Extraction steps omitted 2.60 3.11			5 c.c. was used in these determinations

Note: Each of the above figures is an average of a series of determinations. Since commercial lecithin is impure, it may be wise for comparison purposes to use the result obtained by the Pregl method as 100 per cent and refer all figures to that as a standard. The per cent yields of phosphorus for the several procedures are respectively 67.3, 29.7, 95.1, 95.5, and 98.3 per cent, taken in the order given in the table.

TABLE III  
MG. LIPID P RECOVERED FROM BLOOD BY DIFFERENT METHODS

NUMBER OF BLOOD	BENEDICT- THEIS	BLOOR		KRASNOW- ROSEN
		USING BLOOR EQUIVALENT 1 MG. LECITHIN = 3 C.C. N/10 KIO <sub>3</sub>	USING AUTHOR'S EQUIVA- LENT 1 MG. LECITHIN = 2.8 C.C. N/10 KIO <sub>3</sub>	
364	184	236	253	267
365	190	205	220	242
366	201	211	225	252
367	176	220	232	270
368	230	266	278	315
369	173	212	227	267

Although the results obtained by Bloor's method check those obtained by the Krasnow-Rosen technic quite well, there must be some compensating mechanism involved. As was previously shown, when a solution of commercial lecithin was treated as though it were blood and carried through all the steps in the procedure, the recovery was only 22 mg.  $\approx$  0.86 phosphorus, whereas when the extraction part of the method was omitted, the yield was 2.85 mg. P. It seems then, that other substances are extracted from the blood and when oxidized, they compensate for the loss of alcohol-ether soluble phosphorus.

From our study, thus far, it appears that the oven method is to be preferred for routine work. The results are more accurately reproducible. Although the Zahnd-Clarke technic also yielded similar average figures, the proce-

dure requires more attention, so that for a large number of determinations, the former saves much time, both during the oxidation and during the evaporation of the alcohol-ether extract preparatory to oxidation.

With slight modifications involving volume adjustments, our technic has proved applicable to analysis of lipid phosphorus in spinal fluid and saliva, and total phosphorus in casein. Analyses of amounts lower than 0.1 per cent have been duplicated with a good degree of accuracy. A glance at the data given in Table III will illustrate this.

TABLE III  
DUPLICATE ANALYSES

SUBSTANCE	MG. PER CENT		AVERAGE
Casein	0.78	0.82	0.81
	0.76	0.76	0.76
Spinal fluid	0.024	0.023	0.024
	0.023	0.024	0.024
Saliva	0.125	0.130	0.128
	0.360	0.390	0.375

Furthermore, although several thousand analyses have been made during the last three years the oven used shows no effect of acid corrosion.

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## VITAL STAINING OF MALARIAL PARASITES\*

### A PRELIMINARY NOTE

V. P. SYDENSTRICKER, M.D., AND G. P. VRYONIS, M.D., AUGUSTA, GA.

ALTHOUGH the method to be described undoubtedly is known to many, no reference to it appears in the American or English literature. Malarial parasites stain readily with the so-called vital stains. Boucher<sup>1, 2</sup> has called attention to the usefulness of neutral red and Janus green in the study of the cytology of these organisms. Akashi<sup>3</sup> has also used this method, but his article is not available for reference. For some ten years the senior author has used vital staining with brilliant cresyl blue for demonstration and study of the malarial parasites. While making reticulocyte counts on malarial blood, Vryonis noted that marked changes in the appearance of the parasites and in their distribution in reticulocytes occurred after the initiation of quinine therapy.

Other vital stains have been studied. Neutral red colors the cytoplasm an irregular pink, the chromatin orange red, but the contrast is poor, and the method offers little advantage over unstained wet preparations. With Janus green the parasites stain pale bluish green, the mitochondria a more intense green. With mixtures of these stains such as are used for the study of leucocytes the appearances described are superimposed. With dilute Wright's stain dried on slides and used as a supravital stain, the contrast is exceedingly poor. With all three of the above methods, staining is so faint that it is of little aid except to an observer adept in vital staining and in the study of malarial parasites. Brilliant cresyl blue has the advantages of intense staining with fair differentiation of cytoplasm and nuclear material and of relatively low toxicity for the parasites, though it is more toxic than neutral red. Any technic which gives good reticulocyte staining will give satisfactory results with malarial parasites. The one which has proved most satisfactory in our hands is the following: Using glass prepared for vital staining a small drop approximating 2 c.mm. of saturated solution of brilliant cresyl blue in sterile physiologic salt solution is placed on a slide, a slightly smaller drop of blood is taken on a coverslip and dropped directly on the stain. The preparation is ringed with soft petrolatum. Staining is almost instantaneous, and the parasites remain alive for upward of two hours on the warm stage. Cytoplasm stains irregularly, presenting a coarsely stippled appearance, pale blue with slightly darker areas; chromatin is dark blue; pigment is readily visible.

Marked differences are seen between *P. vivax* and *P. falciparum*. The merozoites of *P. vivax* appear as pale blue pyriform objects with a single dark

\*From the Department of Medicine, University of Georgia School of Medicine.  
Received for publication, January 2, 1935.

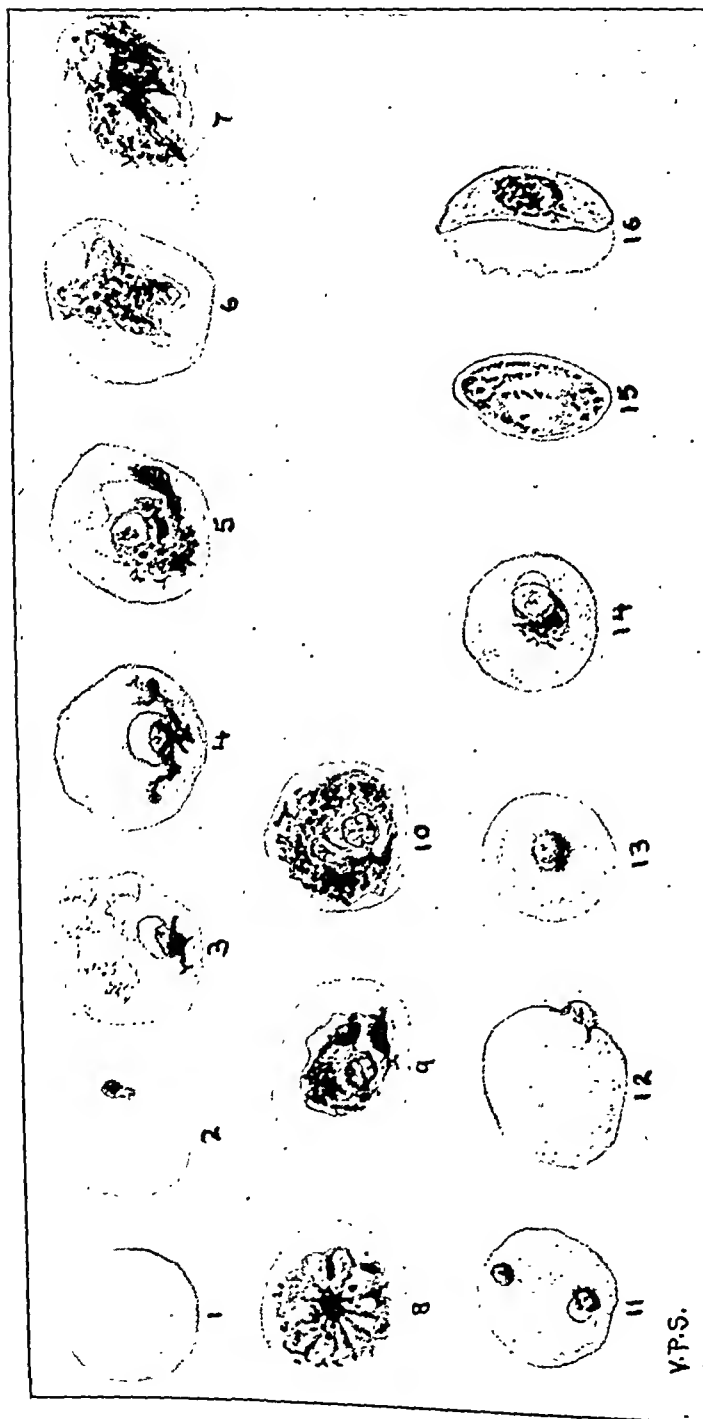


Fig. 1.—Sketched with the camera lucida at magnification approximately 2400. Somewhat reduced in photography. 1, Normal erythrocyte; 2-8, *P. vivax*; 2, microzote on surface of erythrocyte; 3, ten- to twelve-hour "ring form" in reticulocyte; 4-8, stages in development of schizonts; 9, microgametocyte; 10, macrogametocyte; 11-16, *P. falciparum*; 11, macrogametocyte; 12, "marginal" form; 13, typical ring form; 14, twenty-four-hour pigmented schizont; 15, immature microgametocyte ("ovoid"); 16, macrogametocyte.

V.P.S.

blue granule in the smaller end. On attachment to an erythrocyte a pressure halo is visible for some time, and the parasite may be seen to move about on the surface of the cell. After entry to the red blood cell has been effected, an extraparasitic vacuole soon develops, and there is active ameboid movement. The extraparasitic vacuole persists through all stages of development. With age the dye is increasingly absorbed so that tertian parasites over twenty-four hours old are striking objects. Schizonts are easily recognized though no definite nuclear structure can be made out, gametocytes are readily identified, and in favorable optical planes the nucleus is readily seen. All stages of *P. falciparum* stain less intensely. Merozoites are smaller and more rounded, and on attachment to an erythrocyte they soon protrude a single blunt pseudopodium (Fig. 1, 11). After entering a red cell, an extraparasitic vacuole is absent or exceedingly small, and ameboid movement is sluggish. So-called marginal forms show pseudopodia well embedded in the erythrocyte. Twenty-four-hour pigmented forms show many very small pseudopodia and occasionally a minute extraparasitic vacuole (Fig. 1, 14). Mature schizonts have been observed but not under conditions when sketching was possible. The gametes stain characteristically (Fig. 1, 15, 16). Flagellation of microgametocytes has been observed in both species described. No opportunity has been afforded to study *P. malariae*. After death parasites of both species "round up" and show much the same morphology seen in stained dry smears.

After the initiation of quinine therapy in both species, ring forms have been found in nonreticulated erythrocytes only. The significance of this observation and its possible relation to Eaton's<sup>4</sup> hypotheses that the effect of quinine is on the parasites of greatest age and that the erythrocyte is susceptible to infection with malaria only during the reticulocyte stage, are subjects of present study. Under quinine the gametes of *P. falciparum* show striking changes in appearance, staining is more even and intense, the parasites are smaller, and their shape is distorted. Critical studies of the gametocytes of *P. vivax* under therapy have not been made.

The method offers certain advantages for demonstration and for the study of the malarial parasites. The organisms are well stained and easily recognized. They may be observed during life much more readily than in unstained fresh preparations. The morphology of the species under observation is easily differentiated by persons not trained in parasitology. The intracorpuseular location of parasites past the merozoite stage can definitely be demonstrated. It is likely that the method will facilitate study of the relation of parasites to reticulocytes and also of the mode of action of antimalarial drugs.

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## PUNCTURE OF THE INTERNAL JUGULAR VEIN FOR DIAGNOSTIC PURPOSES\*

JUSTIN J. STEIN, M.D., CINCINNATI, OHIO

THE procedure of removing blood from the internal jugular vein is simple and apparently without danger. It would appear that while used in several clinics it might be more widely employed. While it is adaptable to patients of

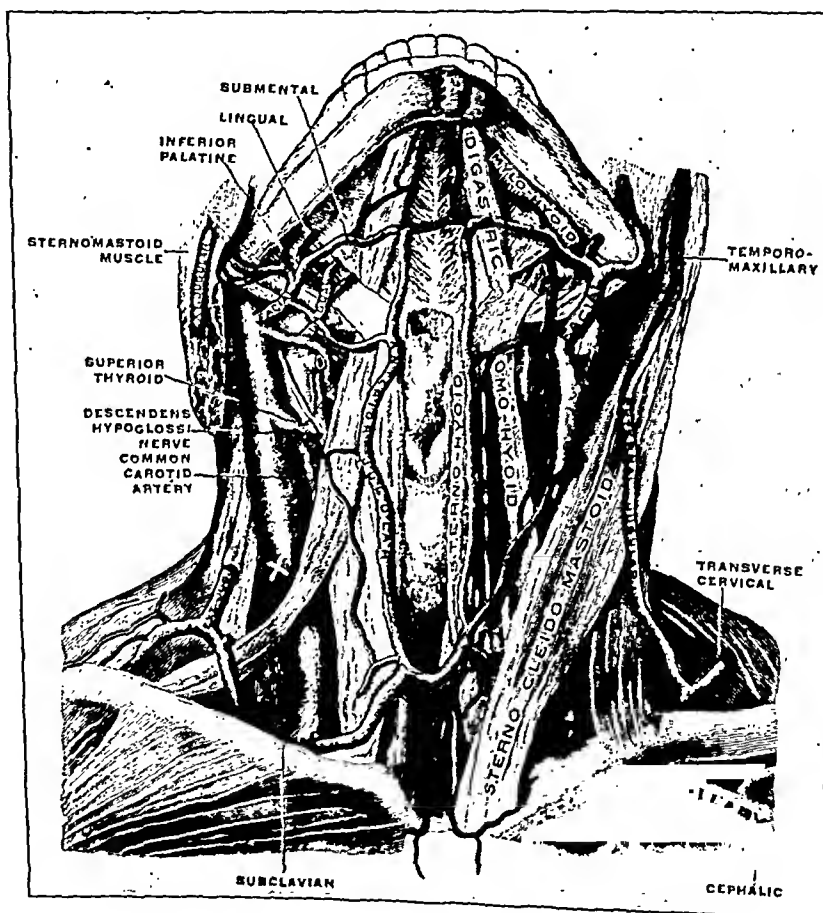


Fig. 1.—Illustration showing internal jugular vein on right, and X represents the point at which the needle enters the vein. (Spaltenholz.)

all ages, it is particularly valuable in infants in whom, especially when they are dehydrated, it is difficult to find superficial veins which are readily accessible for the withdrawal of blood.

\*From the Department of Pediatrics, University of Cincinnati, the Cincinnati General Hospital and the Children's Hospital.  
Received for publication, January 24, 1935.



## TECHNIC

Either internal jugular vein may be used, the right being preferable since it is slightly larger and lies in a more favorable position. The patient is placed on his back with the head lowered over the side of the bed or the end of the table, so as to outline properly the sternocleidomastoid muscle and the external jugular vein. His head is turned as far as possible to the side opposite that on which the vein is to be punctured. The neck is painted with iodine which is subsequently removed with alcohol. The lobe of the ear should be similarly treated. A long needle with a small lumen, preferably a 20 gauge needle 2 inches in length, is attached to a syringe and, with the sternocleidomastoid muscle clearly outlined it is introduced just posterior to the point where the *external* jugular vein crosses this muscle at its posterior border. If the *external* jugular vein is not visible, the needle is then introduced at the junction of the upper and middle



Fig. 2.—Showing the starting point of the insertion of the needle under the sternocleidomastoid muscle. Dotted line represents the direction which the needle takes under the muscle toward the sternal notch.

thirds of the posterior portion of the muscle. When introduced, the needle should be in a position almost parallel with the muscle and posterior to it but *inclined inward so as to point toward the sternal notch*. If the wall of the vein is not punctured when this procedure is followed, the needle may then be introduced almost its entire length, and, while withdrawing it slowly, gentle suction be made until the lumen of the syringe fills with blood.

The anatomical considerations of the internal jugular veins are demonstrated in the illustration. The glossopharyngeal and hypoglossal nerves pass between the artery and the vein; the vagus descends behind the artery and the vein and in the same sheath. At the root of the neck the internal jugular vein unites with the subclavian to form the innominate vein. The nerves are pushed aside if the needle is thrust in their direction, and no harm results. When the

needle is introduced almost parallel with the sternocleidomastoid muscle and kept just beneath it, no other structure will be interfered with. Myerson, Halloran and Hirsch<sup>1</sup> state that transfixing the walls of the jugular vein or of the internal carotid artery is not harmful, since they have repeatedly examined the vessels by open inspection after multiple punctures had been done.

#### CONTRAINDICATIONS

There are apparently no contraindications except infection in the neck or tumor masses in this region.

#### SUMMARY

It is recommended that puncture of the internal jugular vein be used more frequently for diagnostic purposes. In employing this procedure in over 1,000 instances and often in the same subject on several occasions no difficulty of any sort has been experienced.

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<sup>1</sup>Myerson, Halloran, and Hirsch; *Arch. Neurol & Psychiat.* 17: 807, 1927.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

**POLIOMYELITIS, Acute Anterior, Successful Vaccination Against, Kolmer, J. A., and Rule, A. M. Am. J. M. Sc. 188: 510, 1934.**

The vaccine used by Kolmer was prepared as follows:

1. Healthy adult *Macacus rhesus* monkeys, weighing 4 or more kg. of weight, were inoculated intracerebrally (frontal lobe) with 0.2 c.c. of a 5 per cent emulsion of monkey poliomyelitic cord (Rockefeller Institute strain) under ether anesthesia.

2. Symptoms of poliomyelitis usually developed in five to nine days and immediately after death, or when the infection was very severe, the hair was sprayed with 5 per cent trichresol solution and the skin over the head and back removed. The cords were then removed under aseptic precautions after the method of removing spinal cords from rabbits in the preparation of rabies vaccine by the original Pasteur method and 8 gm. of a mixture of several cords thoroughly emulsified aseptically in 100 c.c. of sterile physiologic saline solution.

3. After fine-mesh filtration under aseptic conditions there was added an equal volume of a sterile 2 per cent solution of sodium ricinoleate in saline solution.

4. This gave a 4 per cent suspension of mixed tissue in 1 per cent sodium ricinoleate.

5. The mixtures were well mixed and placed in an incubator at 37° C. for twenty-four hours and then in a refrigerator at 4° to 6° F. for fourteen days with daily shaking for a few minutes.

6. Subcultures were made and the vaccines kept in the refrigerator throughout the year. Even though sodium ricinoleate is of low bactericidal activity, the authors have found that the final concentration of 1 per cent is usually sufficient for sterilizing the vaccines when slight contamination had occurred in the removal of some of the cords and the preparation of the emulsions.

The authors have taken 0.5, 1.5 and 2 c.c. of the vaccine by subcutaneous injection every five days. The injections were accompanied by some stinging pain, especially after the first but less after the second and third injections, which quickly subsided, but there was little or no local reaction except in the 2 c.c. doses when the local reactions were comparable to those produced by the subcutaneous injection of rabies vaccine. Fever and constitutional reactions, however, were not produced, and, while they have found large doses of sodium ricinoleate by intramuscular injection capable of producing some tissue necrosis in rabbits, yet such were not produced in themselves by even 2 c.c. amounts of this poliomyelitis vaccine.

Fortunately for these experiments their blood sera before inoculation were free of antiviral properties, as determined by mixing 0.2 c.c. of serum with 0.2 c.c. of 5 per cent suspension of virus and injecting the mixture, after standing two hours, intracerebrally into two monkeys. Both animals developed poliomyelitis in about seven days.

That the 3 doses of vaccine produced antiviral antibody was shown, however, by repeating the test two weeks after the last dose of vaccine, when two additional monkeys inoculated intracerebrally with a mixture of 0.2 c.c. of serum and 0.2 c.c. of 5 per cent suspension of virus allowed to stand for two hours, remained perfectly well, whereas a control inoculated with virus alone developed paralysis in six days and finally succumbed. These results have shown, therefore, that the vaccine was apparently capable of producing antibody for the poliomyelitis virus and probably sufficient for engendering immunity.

It appears that this is the only effective method for determining whether or not a vaccine is capable of engendering immunity against poliomyelitis in human beings,

because an injection of virus as a test for immunity as done with monkeys is, of course, not permissible, and to vaccinate a group of children to determine over a period of years how many, if any, develop poliomyelitis as controlled by the incidence among a group of unvaccinated individuals would require the immunization of a very large number in the presence of an epidemic. The serum-antiviral test before and after vaccination, however, may be a quicker and more decisive test for acquired immunity, and especially when conducted with children, who are not as likely to have natural antiviral antibody in their sera.

Since these vaccines never produced the slightest evidence of infection in the monkeys or the authors, and believing that the amount of sodium ricinoleate employed effectively reduces the virulence of the virus, they are at present vaccinating a group of children varying in age from eight months to fifteen years. These have been selected after preliminary antiviral tests with monkeys have shown the absence of antibody in the sera. Three doses are being given at weekly intervals with monkey antiviral tests with the sera of some children after the first and second doses and with the sera of all children after the third dose in order to determine the rapidity of antibody production and the number and size of doses of vaccine required.

The first dose for children under three years has been 0.25 c.c. and 0.5 c.c. for older children up to fifteen. By making the first dose quite small in this manner and waiting seven days, opportunity is afforded for antibody production before the second and third doses are administered. Varying amounts are being given in the second and third doses and a subsequent report will soon be made on the results, along with further details on the preparation and standardization of the vaccine. Since the cord of a large-sized monkey will yield about 200 c.c. of vaccine, and taking a total of 3 to 4 c.c. for the immunization of a child, one monkey will yield sufficient vaccine for the immunization of 50 or more children. If subsequent experiments now under way show that it is possible to prepare vaccine of monkey poliomyelitic brain, the yield would be much larger and the cost correspondingly less.

**DEXTROSE TOLERANCE, An Infection, Schmidt, E. G., Eastland, J. S., and Burns, J. H.**  
*Arch. Int. Med.* 188: 3, 466, 1934.

Seventy-three cases of infection have been subjected to the dextrose tolerance test. Among these were 36 cases of pyogenic infections, of which 18 were studied both during and after the infection, 9 of nonpyogenic febrile conditions and 28 of arthritis and rheumatism.

Among the 36 patients with pyogenic infection 29, or 72 per cent, gave distinctly pathologic curves. The highest curves were obtained in cellulitis, the blood sugar on several occasions rising above 300 mg. and usually remaining elevated over three hours after the ingestion of dextrose. Despite such high blood sugar values, far above the generally accepted normal threshold of 180 mg., glycosuria was present in but 4 cases. When the infection had disappeared and the patient was ready to go home a second tolerance test almost invariably resulted in a normal blood sugar curve. Any previous tendency toward glycosuria had now disappeared.

Both streptococcal and staphylococcal organisms were effective in producing disturbances in the carbohydrate mechanism.

The 9 patients with nonpyogenic febrile conditions also yielded abnormally prolonged blood sugar curves.

Among 28 patients with arthritis and rheumatoid conditions 15, or 53.6 per cent, gave diabetic-like blood sugar curves.

The blood sugar values after fasting in pyogenic, nonpyogenic febrile and arthritic conditions were, with few exceptions, well within normal limits.

With 3 exceptions, cases in which obvious renal complications were present, the blood urea nitrogen level was within normal limits during the various infections.

**PROTEIN, Serum, Simple Method of Estimation of, and Its Value in Tuberculosis,** Pagel, W., and Stott, L. B. *Tubercle*, London 15: 454, 1934.

Into six tubes is placed 0.5 c.c. of physiologic solution of sodium chloride. To Tube 1 is added 0.5 c.c. of fresh filtered ox bile, from which 0.5 c.c. of the mixture is transferred to Tube 2. This process is continued up to Tube 5, and 0.5 c.c. of the mixture from Tube 5 is discarded. The last tube being a control tube contains only 0.5 c.c. of physiologic solution of sodium chloride. After this, 0.5 c.c. of 1:7 dilution of the serum to be examined is added to each tube and all tubes are heated until coagulation of the proteins appears in the last tube. The results are recorded immediately, and after twelve hours' standing at room temperature. There are all degrees of coagulation from intensive opacity like milk (+++), less opacity (++), faint transparency (+), gradually falling (+±), to entire clearness (-).

**SILICA, Inhaled, and its Effect on Normal and Tuberculous Lungs,** Gardner, L. U. J. A. M. A. 103: 743, 1934.

Dusts containing silica are preeminently dangerous. Present knowledge will not permit it to be said that only free silica is harmful; possibly some of the silicates will also be incriminated. One silicate, asbestos, produces a characteristic and dangerous type of pulmonary fibrosis. Silica is a tissue poison. In low dilutions it causes nodular fibrosis; in higher concentrations it produces rapid necrosis of cells of all kinds.

Human silicosis begins by damaging the pulmonary lymphatic apparatus and is followed by the development of nodular fibrosis of the parenchyma of the lungs.

Silicosis specifically predisposes to infection with the tubercle bacillus. The mechanism of this action has not yet been determined. It probably consists in some alteration in the soil rather than in changes induced in the infecting organism.

Nonsiliceous dusts localize about the lymphatic trunks and some of them excite the proliferation of small amounts of loose cellular connective tissue. They apparently do not increase susceptibility to tuberculosis.

Nonsiliceous dusts inhaled in combination with silica modify the action of the latter, altering the anatomic characteristics of the lesions and apparently decreasing the susceptibility to tuberculosis.

**TUBERCULIN TEST, Quantitative, As an Index of Tuberculous Activity,** Badger, T. L., and Myers, W. K. *New England J. M.* 211: 241, 1934.

A total of 207 individuals have been tested with measured dilutions of tuberculin to determine what relation, if any, exists between the reaction to such measured dilutions and the nature of the tuberculous process in the lung.

Two controlled groups were studied. One was a group of 161 female nurses in training and the other was a group of 46 tuberculous women.

Measured dilutions of 1:100,000, 1:20,000, 1:1,000 and 1:100 were used throughout. In addition to the tuberculin tests a thorough history, general physical examination and repeated stereoscopic x-ray examination of the lungs were obtained in every case.

All individuals were under observation for from three months to two years.

The individual response to measured dilutions of tuberculin bore no consistent relation to the activity or inactivity of the tuberculous process.

**ENCEPHALITIS, Epidemic, Epidemiology of, St. Louis Type,** Leake, J. P., Musson, E. K., and Chope, H. D. J. A. M. A. 103: 728, 1934.

The type of disease in the St. Louis outbreak was unlike that in the sporadic cases of the Conomo disease but very much like Type B of the Japanese outbreak in 1924, and almost exactly like the Paris, Ill., outbreak of 1933.

The cases were fairly accurately and completely reported.

The case rate for the entire area was 100 per hundred thousand—69 per hundred thousand for the city and 212 per hundred thousand for the county.

There was no predilection by sex or color.

There was a striking increase in both incidence and fatality rates with age.

The fatality rate was higher in the city than in the county.

The incubation period in different cases showed a variation between nine and fourteen days, with possibly wider limits.

There was a notable rarity of multiple cases in the same family and of obvious contagion between cases.

Between communities the spread was obviously by human contagion; but as regards individuals, individual susceptibility, in which age played a part, appears to be more important than contagion.

The disease appears to be limited seasonally in its typical form.

Water supply and milk supply were eliminated as possible mediums of transmission.

Entomologic experiments with the mosquito as a possible vector were negative.

**GASTRIC ANALYSIS, Detection of Free Acid in Patients With Suspected Anacidity, Necheles, H., and Scheman, L. J. A. M. A. 103: 107, 1934.**

The authors comment that free HCl will combine quickly with mucus thus leading, under the usual methods of gastric analysis, to reports of its absence even though actually it may be present in small amounts associated with an excess of mucus.

The apparatus described below permits a determination of acidity as soon as the sample passes out of the stomach.

It consists of a glass T-tube, the vertical arm of which is wide enough to hold a stopper with a bore. The lower end of a buret is used, the glass tube below the stopcock being pushed through the bore of the stopper. The opening of this glass tube is made as narrow as possible. One end of the T-piece is connected to a suction device with a mercury manometer and needle valve. The other end of the T-tube is connected to the stomach tube. The container above the stopcock is filled with Topfer's reagent. Small sealed glass tubes with various concentrations of hydrochloric acid and Topfer's reagent may conveniently be attached alongside the horizontal part of the T-tube to read the concentration of free acid in the gastric juice. The base of the wooden stand on which the T-tube rests is painted white. As the suction works and gastric contents appear in the T-tube, the stopcock is opened, permitting a drop of reagent to come in contact with the stomach juice, and notes are taken of the color reaction. Mucus, bile and pure stomach juice can thus be tested separately before neutralization takes place.

**ANEROBES, Method for Plate Cultures of, Trambusti, B. Diagn. E. Techn. di Lab. 5: 23, 1934.**

The method following permits the isolation of anaerobes on plates and examination of the colonies without destruction of the surface of the medium.

Three pieces of glass rod or tubing (sealed at the ends) are cut of a length slightly less than the diameter of the Petri dish to be used and arranged on the inverted lid in the form of an equilateral triangle. The medium is added and then the inverted bottom of the dish placed upon the glass triangle. The space between the lid and bottom is then sealed as usual and incubated.

**GONORRHEA, Treatment of Acute by Means of a New Gonococcic Vaccine of Low Toxicity, Price, I. N. O., and King, A. J. Brit. M. J. 1: 748, 1934.**

The authors report favorably upon a gonococci vaccine prepared as follows:

A forty-eight-hour growth of gonococci (hydrolytic agar, pH 7.5, in a triangular Roux bottle incubated at 37.5° C.) is washed off into a cylinder with 100 c.c. of physiological salt solution, giving a suspension containing about 180 million organisms per cubic centimeter.

After the addition of 1 c.c. of N/10 NaOH the cylinder is placed in a 37.5° C. water-bath for two hours to dissolve the gonococci. The fluid is then filtered through sterile lint, 1.5 c.c. of N/10 HCl added. After twenty or thirty minutes the white floccules

appearing are removed by centrifuging (3,000 r. p. m.) and suspended in 9 c.c. of sterile normal saline. To this suspension N/10 NaOH is added drop by drop until the floccules go into solution (pH 7.5). When this occurs 1 c.c. of 1 per cent formaldehyde in normal saline is added.

This solution constitutes the vaccine, 1 c.c. containing the protein of approximately 180 million organisms.

Subcutaneous injections are given daily into the gluteal muscles, starting with 0.25 c.c. and increasing to a maximum of 1.5 c.c. according to the reaction produced.

The vaccine is most effective in chronic gonorrheal conditions but appeared to lessen the incidence of complications when given during the acute stages.

**CYANIDE POISONING**, Gettler, A. O., and St. George, A. J. *Am. J. Clin. Path.* 4: 429, 1934.

The authors recommend the following procedures in the study of cyanide poisoning:

If the poison has been taken by mouth, the stomach contents and brain should be analyzed. Analysis of the brain is necessary for the purpose of ruling out the possibility of the poison having been introduced into the stomach after death. If the poisoning resulted from inhalation, the lungs and brain must be examined. In cases of poisoning by inhalation, usually none, or only the very faintest trace, is found in the stomach contents. This is of tremendous importance from the medicolegal aspect.

#### METHOD OF ISOLATION OF CYANIDE

The tissues are cooled by keeping them in an ice box. Two hundred to 500 gm. of tissue are ground up. Care should be taken to keep the tissue cold since hydrocyanic acid may volatilize if warm. If stomach contents are analyzed, usually one-fifth of the total volume of the contents is used. The ground-up tissue or the stomach contents are placed in a one liter flask and acidified with tartaric acid. The material is then distilled with steam, using a well-cooled condenser the tip of which is bent to serve as an adapter and dipped into 5 c.c. of 5 per cent sodium hydroxide solution in a receiving flask. The latter should be packed in ice. One hundred cubic centimeters of distillate are collected, which is ample to recover all the cyanide present, the following tests being employed:

#### QUALITATIVE TESTS

1. *Schonbein's Test*.—Suspend a strip of filter paper, impregnated with guaiac and copper sulphate, over the material in a flask, the paper being held in place by the stopper. (Dip strip of filter paper into a freshly prepared alcoholic solution of guaiac 1:10; then let dry; when dry, moisten it with dilute (1:10,000) copper sulphate solution.) If color does not change, cyanide is absent and no further tests need be made. If a blue color results, cyanide may be present. The test is very sensitive but not specific; hydrochloric acid, nitric acid, chlorine, bromine, ozone, hydrogen peroxide, as well as some other substances also give a positive test. This test may be used as a preliminary one at the necropsy table. The following two tests must be employed since they are specific for cyanide:

2. *Prussian Blue Test*.—To 5 c.c. of distillate, add 3 c.c. of 25 per cent sodium hydroxide, then a few drops of freshly prepared ferrous sulphate solution and a few drops of ferric chloride solution. Warm a little. Let cool and add concentrated hydrochloric acid, dropwise, until the dirty brown precipitate just dissolves; avoid excess hydrochloric acid. If cyanide is present, a deep blue precipitate (Prussian blue) appears. If only a trace of cyanide is present, a green solution results instead of a blue precipitate, but, on standing several hours, a small flocculent Prussian blue precipitate settles (sensitive to one part in 50,000).

3. *Liebig's Test*.—To 10 c.c. of distillate, add 1 c.c. of yellow ammonium sulphide and evaporate to dryness on the water-bath. When dry, add 5 c.c. of 5 per cent hydrochloric acid solution, warm a little and stir well to dissolve all of the thiocyanate that was formed

during the evaporation. Let stand two hours, then filter. To the filtrate, add 5 to 10 drops of 10 per cent ferric chloride solution. If cyanide is present, a deep red color results (sensitive to one part in 10 million).

The following tests may be used, but they are not specific for cyanide:

4. *Fortmann's Test*.—To 5 c.c. of distillate, add a few drops of potassium nitrite solution, then 2 to 4 drops of ferric chloride solution and then enough dilute sulphuric acid until the color of the solution becomes a bright yellow. The solution should then be boiled, after which cool and add ammonium hydroxide until all of the iron is precipitated. Filter off the precipitate. To the filtrate add a few drops of a very dilute solution of ammonium sulphide. If cyanide is present, a play of colors results, violet, blue, green, yellow.

5. *Picric Acid Test*.—To 5 c.c. of distillate (slightly alkaline) add a few drops of picric acid solution and warm gently. If cyanide is present, a red color develops (sensitive to one part in one million).

6. *Phenolphthalin Test*.—To 5 c.c. of distillate, add a few drops of alkaline phenolphthalin solution (reduced phenolphthalein), then a few drops of 1:2,000 copper sulphate solution. If cyanide is present, a red color develops (sensitive to one part in 20 million).

7. *Silver Test*.—To 2 c.c. of distillate, add nitric acid until reaction is acid, then add a few drops of silver nitrate solution. If cyanide is present, a white precipitate of silver cyanide results.

#### QUANTITATIVE TEST

For quantitative analysis, a weighed amount of tissue is distilled, as described in the qualitative procedure. In the receiving flask, however, instead of having dilute sodium hydroxide, 20 c.c. of 10 per cent silver nitrate solution are used, acidified with nitric acid. In order to make certain that all of the cyanide is isolated, distillation is continued until 200 c.c. of distillate are obtained. During the distillation, the cyanide precipitates as silver cyanide. This is then filtered through a previously weighed Gooch crucible, washed, dried and weighed. From the weight of the silver cyanide, the amount of cyanide in the material analyzed is calculated as HCN.

In quantitative analysis, if the poison was taken by mouth, the entire amount of cyanide is determined in the gastrointestinal tract. This is then multiplied by 100/98, which gives the amount in the entire body. This fraction is used because approximately 98 per cent of the cyanide, if taken by ingestion, remains within the stomach contents. If the poison was introduced by inhalation or injection, parts of all the organs and tissues are analyzed and, from these results, the amount present in the entire body is calculated.

The lethal dose of cyanide is accepted as 50 mg., calculated as hydrocyanic acid.

The following factors interfere with the determination of the presence of cyanide:

1. Traces of hydrogen cyanide are produced during the first days of putrefaction, but this disappears in the later stages.

2. Cyanides present in the tissues disappear during prolonged putrefaction and are changed to sulphocyanide.

3. In the stomach contents, where the bulk of the cyanide remains at death, putrefaction is of little importance.

4. Embalming with formaldehyde interferes greatly in the tests, the cyanide forming condensation products with the formaldehyde.

**SEDIMENTATION TESTS, Repeated.** Van Antwerp, L. D. *Am. J. Dis. Child.* 48: 814, 1934.

The observation that sedimentation rates in children show an unexplained tendency to vary widely without intervening clinical causes should be of value to pediatricians, regardless of the cause. That this occurs commonly is demonstrated by the fact that 27 per cent of the author's patients had readings erroneous enough to mislead in their interpretation. His experience, however, shows that a repeated test will usually give the correct reading. Occasionally a repeated test will show a persistently high rate, though this is not common. In



such an event, careful clinical search should satisfy one as to the presence or absence of any condition warranting a high rate. A persistently high rate need serve, therefore, only as a warning of the possibility of the presence of a pathologic condition; it need not be held as conclusive.

Two sedimentation tests are done on successive days on all newly admitted patients, the lower rate being taken as the one reflecting the presence or absence of a destructive lesion. Van Antwerp is being forced to the conclusion that apprehension on the part of the patient is a factor in erroneous results in sedimentation tests, though he has not thus far been able to prove it. If such is the case, it will be necessary to repeat tests on patients giving a high rate as basal metabolic tests are now repeated. It is already well known that certain types of insanity are characterized by high rates of sedimentation and it is interesting to consider the possibility that the factor influencing the rate in this class of patients may be that of apprehension, because of emotional instability.

Later observations comparing blood sugar content with rates of sedimentation will be offered as a factor of proof or disproof of the contention that apprehension influences the rate of sedimentation.

**EOSINOPHILIA, Rapid Method for Determination of, Freedman, T. B. J. A. M. A. 103: 1618, 1934.**

In place of the usual acetic acid-diluting fluid, the following fluid, which is the modification of Camara and Alvarez of that described by von Douarus, is employed:

Aqueous eosin 1 per cent	5 c.c.
Acetone	5 c.c.
Distilled water	100 c.c.

The count is made in the usual manner in the ordinary counting chamber. This diluting fluid stains only the eosinophilic cells and they are easily distinguishable with the high dry power of the microscope. The unstained leucocytes appear as gray bodies in this diluting fluid.

It is advisable to fill also a pipette with the ordinary acetic acid-diluting fluid and use this count as a check until one has had some experience with the fluid described.

**CHORIONEPITHELIOMA, Early Diagnosis By Quantitative Determinations of Pituitary-Like Principle From the Urine of Pregnancy, Leventhal, M. L., and Saphir, W. J. A. M. A. 103: 668, 1934.**

The quantitative determination of the gonadotropic substance in the urine is an important biologic test in the early diagnosis of chorionepithelioma.

Following the expulsion of a hydatidiform mole, this quantitative test forms a prognostic index for chorionepithelioma formation.

In the absence of clinical manifestations, an amount of gonadotropic substance in the urine in excess of 20,000 mouse units per liter indicates an early chorionepithelioma.

A case of early chorionepithelioma was diagnosed and an operation was performed solely on the basis of the laboratory finding of 333,000 mouse units of gonadotropic substance per liter of urine in a patient who had expelled a hydatid mole four and one-half months previously.

Recurrence of chorionepithelioma or metastases may be discovered by the Aschheim-Zondek reaction, and this should determine the use of radiation therapy.

## REVIEWS

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Books and Monographs for Review should be sent direct to the Editor,  
Dr. Warren T. Vaughan, Professional Building, Richmond, Va.

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### Definite Diagnosis In General Practice\*

**A**S DR. MUSSER remarks in the foreword to this book, "there is no quick road to diagnosis," and the importance of diagnosis in the management of disease requires no comment.

In constructing his book Dr. Kitchens has chosen, from among the diseases to which the human race is heir, 407, representing those most likely to be encountered in the most wide and varied practice; and from his own experience as well as from a thorough study of medical literature, has selected 506 symptoms of definite diagnostic importance.

The book is planned for use as a quick reference, as a simplified means of differential diagnosis, and as a method assisting in selective or eliminative diagnosis, or, as often called, diagnosis by exclusion.

The volume is divided into two sections.

Part I includes, first, a Symptom Index which presents in alphabetical order 506 symptoms of 407 diseases, each symptom being preceded by the page number on which that symptom is found.

Following the Symptom Index each symptom is presented on a separate page and under each symptom are listed those diseases in which that symptom is of important diagnostic significance. Furthermore, each disease is preceded by the page number in Part II on which the full symptomatology of that disease is found.

Part II presents, first, 506 diseases, one to a page, under each of which are presented those symptoms of diagnostic significance, giving first Subjective Symptoms, then Objective Symptoms, and finally Laboratory Findings. Each symptom is preceded by the page number in Part I where that symptom is listed.

Following the presentation of diseases, there is a Disease Index listing in alphabetical order the 506 diseases, each disease being preceded by the page number in Part II where the full symptomatology of that disease is found.

If it is desired to review quickly the symptomatology of any disease, the reader turns to the disease in Part II. Or, given a symptom, if it is desired to learn at a glance those diseases in which that symptom is found, the reader turns to the symptom in Part I.

To differentiate quickly any two or more diseases, the reader turns to those diseases in Part II and checks the complete and possible symptomatology of one against the other.

The book provides ample space for the addition by the reader of observations reflecting his own experience or the observations of others so that it may be kept continually up to date.

The book is simple in its method of use and should prove without doubt, as it is intended to be, of definite aid in the formation of a diagnosis.

### Statistical Methods for Research Workers†

**T**HIS volume is one of a series of Biological Monographs and Manuals issued under the editorship of F. A. E. Crew.

The purpose of the present volume is to present to research workers the practical procedures relative to statistical methods which are appropriate to their problems and for the presentation of their results.

That the book has reached its fifth edition testifies to its usefulness.

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\*Definite Diagnosis In General Practice. By W. K. Kitchens, M.D. Cloth, pp. 1000. W. B. Saunders Co., Philadelphia, Pa.

†Statistical Methods for Research Workers. By R. A. Fisher, S.C. Formerly Fellow of Oliver and Boyd, Edinburgh, etc. Cloth, ed. 5, pp. 319, 6 insert tables, 68 text tables.

# The Journal of Laboratory and Clinical Medicine

VOL. 20

JULY, 1935

No. 10

Editor: WARREN T. VAUGHAN, M.D.

Richmond, Va.

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## EDITORIALS

### Etiology of Granulopenia (Agranulocytosis)

EVER since Schultz<sup>1</sup> in 1922 described as a clinical entity the association of gangrenous throat lesions with extreme leucopenia, there has been an increasing interest in the condition and much speculation concerning its etiology. It is now known that the primary change is found in the bone marrow and that the granulopenia is only a reflection of the marrow dysfunction. The necrotic local lesions result in turn from the loss of the defense normally provided by the neutrophilic leucocytes. Often there is no superficial necrosis even with a marked leucopenia, although weakness is a very constant symptom, and it is usually accompanied by chills and fever.

In his cases, Schultz found a marked aplasia of the myeloid cells in the bone marrow with little involvement of erythrocytes or platelets. Further studies of the marrow of patients dying from granulopenia with or without mucosal lesions have revealed widely varying findings. Often the marrow is

hyperplastic rather than aplastic, but there is always some disturbance in the state or rhythm of development of the granulocytes. Fitz-Hugh and Krumbhaar<sup>2</sup> emphasize the lack of maturation of the neutrophils even when there is an abundant supply of immature myeloid cells, and they suggest that the disease is fundamentally a "maturation arrest" of the granulocytes. Jaffé<sup>3</sup> found striking qualitative changes in the myelocytes in the marrow which were evidenced by marked degenerative changes. It is apparent that the marrow picture must vary with the stage of the disease. In the early stage, there may be only a disturbance in maturation; later, the myelocytes show evidence of an intoxication; and still later, the myeloid tissue may be aplastic. With massive dosage of the agent responsible for the marrow damage or a hypersusceptibility of the patient to small doses, extreme marrow degeneration may occur early in the course of the disease. Thus, it is apparent that the marrow picture is the primary and important one. The change is essentially a myelotoxicosis which varies greatly in degree and intensity. At times the clinical picture of the disease is seen with some known disorder of the marrow, such as aplastic anemia, leukemia, or myeloma. Here the cause of the clinical picture is the same as that responsible for the cryptic cases, although the bone marrow disturbance is different from that seen in idiopathic granulopenia.

The most perplexing problem has been the etiology of the marrow injury. Radiant energy may cause a marked depression of the marrow, but this can usually be ruled out as a possible causative factor. Overwhelming infection may affect the marrow in a similar manner and cause a granulopenia. However, such an infection usually is apparent as the primary disease. The local lesions of granulopenia show a great variety of organisms and the blood culture is often positive late in the disease. No organism, however, has been proved to be constantly and etiologically related to the disease. It is possible that a relatively mild infection may further depress a hypoplastic marrow and produce a granulopenia.

The possibility of a chemical cause for the disease has always been intriguing. It has long been known that certain chemicals will cause an aplasia of the bone marrow with involvement of all marrow elements. Benzene is the classic example of such a drug. It is interesting that most other drugs which have a similar action, such as arsphenamine, also contain the benzene ring. This has been emphasized by Kracke.<sup>4</sup> In granulopenia we have a marrow disturbance similar to that caused by benzene except that there is selective involvement of myeloid tissue rather than simultaneous involvement of myeloid, erythroblastic and platelet-forming tissues. It is true, however, that in granulopenia there is usually some involvement of red cells and platelets, although in a much less degree than in aplastic anemia. A most important contribution to the etiology of the disease is the recent demonstration that amidopyrine and similar commonly used drugs may produce the disease. Kracke<sup>4</sup> noted that eight of nine patients with granulopenia of undetermined origin had been taking drugs to affect the leucocyte count experimentally with such drugs, although he was able to do so with the oxidation products of benzene. Madison and Squier<sup>5</sup>

were the first to offer convincing proof, largely from clinical observation, that amidopyrine alone or in combination is a definite cause of granulopenia. Two of their patients, after recovering from an attack, again developed a profound granulopenia when a single dose of amidopyrine was taken. Sturgis and Isaacs<sup>6</sup> report similar observations. Evidence is rapidly accumulating<sup>7, 8, 9</sup> that amidopyrine and similar drugs are the most important etiologic agents in idiopathic granulopenia. Kracke<sup>10</sup> in reviewing the relationship of drugs to the etiology of the disease concludes that drugs containing the benzamine group (amidopyrine, phenacetin, acetanilid, arsphenamine, and neorsphenamine) are most likely to cause the disease.

The drugs which have been so incriminated are very commonly used. The question arises why they relatively seldom cause trouble. Pepper<sup>11</sup> has pointed out the frequency of allergy and drug sensitization in cases of granulopenia. This is a most important observation because it seems that, in most cases, the sensitivity of the patient determines the toxic effect of a small dose of a drug which has a specific affinity for myeloid tissues. This also well explains the difficulty in reproducing the disease experimentally. In some cases the disease is due to large doses over a long period of time with cumulative action or development of sensitivity by reason of prolonged use. At times the marrow may be already hypoplastic making it more susceptible to further injury.

The etiology of granulopenia thus seems solved in the demonstration of drug relationship to the disease. Time will probably prove that most cases of so-called idiopathic granulopenia are due to the toxic action of some drug on the marrow either by reason of the hypersensitivity of the patient or overdosage of a drug with a selective action on normal or hypoplastic myeloid tissue.

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—R. L. H.

# The Journal of Laboratory and Clinical Medicine

VOL. 20

AUGUST, 1935

No. 11

## CLINICAL AND EXPERIMENTAL

### FURTHER OBSERVATIONS ON PATIENTS WITH HIGH BLOOD SUGAR BUT NO GLYCOSURIA\*

RALPH H. MAJOR, KANSAS CITY, KAN.

IN 1925 Major and Davis<sup>1</sup> described eight patients suffering from diabetes mellitus who were under treatment with insulin and showed a high blood sugar with no sugar in the urine. The ages of these patients varied from eleven years to seventy-two years and height of the blood sugar elevations from 196 mg. per 100 c.c. to 425 mg.

#### FOLIN-WU METHOD

NO.	AGE	BEFORE FERMENTATION	AFTER FERMENTATION	URINE
1	26	500 434 444	71 36 19	Neg. Neg. Neg.
2	63	189	36	Neg.
3	35	200	18	Neg.
4	51	230 218	28 30	Neg. Neg.
5	56	247 200	30 24	Neg. Neg.
6	65	333	32	Neg.
7	58	227	33	Neg.
8	20	363	28	Neg.
9	17	250 307 238	34 36 18	Neg. Neg. Neg.
10		267	28	Neg.
11	20	244	20	Neg.
12	59	303 500 236	42 41 28	Neg. Neg. Neg.
13		207	32	Neg.

\*From the Department of Internal Medicine, University of Kansas School of Medicine.  
Received for publication, December 17, 1934.

The observations of Somogyi<sup>2</sup> showing that a part of the substance in the blood which reduces copper sulphate is not glucose since it does not ferment, suggested a further study of patients showing high blood sugars but no sugar in the urine. It seemed theoretically possible that the apparent high blood sugars might be due to the presence in the blood of substances other than sugar, which could not be excreted in the urine. This question has been studied further in sixteen patients who showed high blood sugar but no sugar in the urine. In these patients the blood and urine specimens were collected simultaneously.

The blood filtrates were prepared according to the method of Somogyi. Sugar determinations were carried out in one series of observations by the Folin-Wu method and in the second series by the Shaffer-Hartmann method.

## SHAFFER-HARTMANN METHOD

NO.	AGE	BEFORE FERMENTATION	AFTER FERMENTATION	URINE
1	45	241	39	Neg.
		274	34	Neg.
		237	44	Neg.
2	59	172	34	Neg.
3	30	201	41	Neg.
4	49	183	31	Neg.
5	48	183	39	Neg.
6	51	359	34	Neg.
		203	23	Neg.
		279	29	Neg.
7	63	333	31	Neg.
		200	29	Neg.
		196	18	Neg.
8	66	196	18	Neg.
9	55	194	29	Neg.
10	51	176	36	Neg.
11	77	260	34	Neg.
12	10	203	31	Neg.
13	64	225	39	Neg.
14	20	241	29	Neg.

These observations show that the high values for the blood sugar in these patients are due to a fermentable sugar, presumably glucose, and not to any other nonexcretable substance. The absence of sugar in the urine is evidently due to what is commonly termed a high renal threshold for sugar.

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## THE ANTISEPTIC AND BACTERICIDAL ACTION OF UREA\*

JOHN H. FOULGER, M.D., AND LEE FOSHAY, M.D., CINCINNATI, OHIO

UREA, historically significant as the foundation stone of modern synthetic organic chemistry, is generally regarded as nothing more than a convenient and, perhaps, from the standpoint of energy, an economic form in which the mammalian organism can excrete nitrogen. Yet a close search of the literature reveals isolated reports of biologic activities of urea which may be of considerable practical value.

In an account of the action of urea upon proteins, Ramsden<sup>1</sup> made the very interesting observation that urea prevents putrefaction. Such an action, it appeared to us, could be due either to the suppression of enzyme activity or, more important still, to the inhibition of growth of those organisms which fabricate proteolytic enzymes. Experiments by one of us (J. H. F.) show definitely that enzyme action may be retarded by low concentrations of urea. The present paper reports results obtained by the use of saturated solutions of urea in the treatment of purulent exudates and data on the bactericidal action of urea (by L. F.).

The first detailed study of urea as a bactericide is that of Péju and Rajat.<sup>2</sup> Following the observations of other workers, whom they do not specifically mention, these authors studied the polymorphism produced in bacteria by urea. They found that on media containing urea there was less and less bacterial growth until media saturated with the compound remained sterile. Coincident with the decrease in growth there was, in some cases, an increase in the number of abnormal morphologic forms. There were present sometimes long, empty, unbranched filaments of uniform, homogeneous structure and sometimes large oval or pyriform masses or filaments with such masses at the extremities or occasionally on their length. Ultimately, and after a period characteristic for each organism, the bacteria, if left in the urea medium, tended to revert spontaneously to their normal forms. According to the ease with which they were affected by urea, the organisms studied were divided into three classes:

I	II	III
<i>B. Eberth</i>	<i>B. cholerae</i> (Calcutta)	<i>B. violaceus</i>
<i>B. diarrhoea</i> (green)	<i>B. Thollér</i>	<i>B. septicus aerobius</i>
<i>B. psittacosis</i> (Nocard)	<i>B. butylicus</i> (Binot)	<i>B. mycoides rosaceus</i>
<i>B. pyocyaneus</i>	<i>B. Korn 1</i>	<i>B. anthracis</i>
<i>B. dysenteriae</i> (various types)	<i>B. Gross</i>	<i>B. diphtheriae</i>
<i>B. enteritidis</i> (Gartner)	Mistbacillus	Various cocci (staph., strep., pneumo., sarcinae)
	<i>B. tuberculosis</i> (human)	

\*From the Departments of Pharmacology and Experimental Bacteriology, University of Cincinnati.

Received for publication, February 4, 1935.



The bacteria in Group I were easily modified by urea, polymorphism being produced readily and rapidly. Those in Group II were not easily modified, often requiring several weeks for a slight change. The organisms in Group III showed no polymorphism in presence of urea. No information was given which would allow of correlation of polymorphism and growth in media of different urea strengths.

Wilson<sup>3</sup> found that 8 per cent urea prevented the growth of *B. coli*, while 1.5 to 3.5 per cent caused polymorphism.

No great attention was paid to the bactericidal action of urea until Symmers and Kirk<sup>4</sup> reported bacteriologic and clinical studies. They found a number of interesting and important facts, which can be summarized as follows:

a. Urea prevents bacteria from deoxidizing blood.

b. No growth could be obtained on tubes inoculated from twenty-four-hour agar culture of *B. typhosus* which had been subjected for thirty minutes to the action of a saturated solution of urea.

c. An old, putrid, tuberculous sputum, which gave, on agar, confluent growths of various organisms, was treated at room temperature with a saturated solution of urea. After fifteen minutes a loopful was smeared on agar. Only three colonies grew. After thirty minutes' action of urea only one colony grew.

d. Five cubic centimeters of blood were inoculated with *B. pyocyaneus*. Controls gave abundant growth, but transplants from the mixture after it had been treated with 2.5 gm. of urea for thirty minutes at room temperature were sterile.

e. A similar mixture containing 1.25 gm. of urea gave ninety-eight colonies on an agar transplant after standing fifteen minutes, while after standing thirty minutes only five colonies grew.

f. Below a concentration of 25 gm. of urea per 100 c.c. of blood there was no definite bactericidal action, but growth was delayed.

Symmers and Kirk then proceeded to clinical tests of the bactericidal action of urea. At first they used a fresh 100 per cent aqueous solution. This they found of undoubted value as a wash in the treatment of diphtheria carriers. In the treatment of wounds closer and more continuous contact with urea was needed. Therefore the infected tissues were liberally dusted with the powdered compound and the wounds finally closed with continuous sutures to prevent drainage. That urea is innocuous to human tissues was adequately proved. Thiersch epithelial skin grafts over healthy ulcers never necrosed in presence of urea. A simple, oblique fracture of the shaft of the femur, in a young man, was treated with 6 gm. of powdered urea, which was placed alongside the fracture after plating. The muscles, fascia lata, fat and skin layers were closed separately with continuous sutures. There was no sign of interference with healing, and in three weeks all splints and dressings were discarded and massage and passive movements commenced. In many radical operations for inguinal hernia, urea was placed between the different tissue layers without damage. In one case with a chronic staphylococcus blood infection, urea was sprinkled between the layers of tissue and the wound then closed with continuous sutures. Healing followed with no sign of infection. Sloughing, infected wounds, dressed with urea powder once in twenty-four hours, gave better results than similar wounds treated by other methods. The absence of venous congestion in tissues was

noticeable in contrast with cases treated with ordinary wet dressings, continuous irrigation, or frequent baths. The circulation was normal and repair seemed more rapid. There was no irritation.

The only disadvantage noted with the urea treatment was occasional severe pain, which, however, could be controlled by morphine. Since urea absorbs moisture rapidly, the powdered solid easily became caked. This caking was prevented by covering the urea layer with oiled silk and the latter with a dry dressing. The wounds required dressing only once in forty-eight hours.

Unaware of the work of Symmers and Kirk, one of us (J. F.) selected as material for a clinical study of urea a few cases of purulent otitis media, complicating contagious diseases in children. All the patients studied were on the Pediatric Service of Cincinnati General Hospital. In most cases the primary disease was scarlet fever and the ears were infected with a hemolytic streptococcus. Also, most of the cases had failed to respond to treatment with mercurochrome, hydrogen peroxide, silver preparations, etc. The technic used was simple. A fresh, saturated aqueous solution of urea was instilled into the affected ear every four hours, using a medicine dropper. Since it was soon found that the urea crystallized out and plugged the external canal, thus hindering drainage and preventing observation, the ear, before each instillation of urea, was gently irrigated with normal salt solution, again using a medicine dropper.

The number of cases treated (20) is too small for statistical study. The results of the treatment were in general as follows: All of the cases which had failed to respond to other local medicaments responded to urea. The discharge, often of a foul odor, lost its foulness in a few hours and became serous or mucoid. In from three to six days all discharges ceased. Smears from ears which originally contained hemolytic streptococci no longer showed this organism, but often a hemolytic staphylococcus not previously present was found. There were no signs of irritation.

Cases treated with urea at first onset of otitis media almost all responded rapidly, sometimes clearing up after thirty-six hours and usually within three to six days. Twice discharges were checked but recurred a week or two later, after urea treatment was stopped. In both cases a mastoiditis was present and was later treated by radical operation.

Two results of urea treatment are of special interest. The first patient, a boy of ten years, developed a bilateral otitis media and a hemorrhagic nephritis about the third week of hospitalization with scarlet fever. The ear condition failed to respond to mercurochrome, hydrogen peroxide, and silver preparations. Macroscopic and occult blood were present in the urine, which was examined daily. After three weeks of this condition urea treatments were started. The ear discharges at once became less foul and more serous. At the same time the blood gradually disappeared from the urine. After ten days of urea treatment only a slight serous discharge was present and tests for blood in the urine were negative. The discharge never entirely ceased until mastoid operations were performed.

The second patient, a girl of five years, was admitted with a severe streptococcal throat infection. The nose and submaxillary lymph nodes were soon involved. The latter were opened and drains inserted. In spite of persistent

treatment with potassium permanganate, hydrogen peroxide, and other preparations, the infection spread. The patient became delirious and finally comatose. The foul odor of discharges from the nose, mouth, and lymph nodes permeated the ward. When no other treatment availed and the child was moribund it was decided to use urea. The nose and throat were irrigated with a saturated solution of urea every two hours. The draining lymph nodes were packed with gauze soaked in a saturated solution of urea. Within twenty-four hours the odor of the discharge was diminished and had disappeared in three days. The patient died a week later from peritonitis.

One of us (L. F.), interested in these clinical results, has again tested the bactericidal action of urea.

Fresh twenty-four-hour cultures of thirteen common species were washed from appropriate mediums in exactly 5.0 c.c. of sterile saline and tubed. The turbidity of each suspension was measured against the usual fuller's earth standard. Then to each tube was added exactly 5.0 c.c. of a freshly prepared saturated solution of urea in distilled water and the suspension thoroughly mixed. The tubes stood at room temperature throughout the entire period. After two hours of exposure to half-saturated urea, 1.0 c.c. was withdrawn from each tube and transferred to other tubes containing 15 c.c. of sterile saline. All tubes were centrifuged until the supernatants were clear. The bacteria were washed once in saline, and the entire final sediments were transferred to appropriate mediums to determine viability. This procedure was repeated at three hours and again at four hours of exposure. After twenty-four hours the original urea-bacteria suspensions were washed twice with saline and large loopfuls of the final heavy sediments were similarly transferred to appropriate mediums. Meat extract broth was used for the hardier species; dextrose ascites broth for the streptococci and staphylococci; and dextrose cystine ascites hemoglobin agar for *B. tularensis*. All culture tubes were incubated at 37° C. for five days before discarding. The viabilities of these species after exposure to half-saturated urea are shown in Table I.

TABLE I  
VIABILITY AS DETERMINED BY CULTURE

SPECIES USED	TURBIDITY OF ORIGINAL SUSP.	EXPOSURE TO HALF-SATURATED UREA			
		2 HR.	3 HR.	4 HR.	24 HR.
<i>B. coli communis</i>	3000	0	0	0	0
(2 strains)	3000	0	0	0	0
<i>B. coli communior</i>	3000	0	0	0	0
(2 strains)	3000	0	0	0	0
<i>Staph. aureus</i>	2000	+	+	+	+
<i>Staph. albus</i>	2000	+	+	+	0
<i>Strep. fecalis</i>	800	0	0	0	+
(2 strains)	800	0	0	0	0
<i>Strep. viridans</i>	100	0	0	0	0
<i>B. mucosus</i>	2500	0	0	0	0
(2 strains)	2000	0	0	0	0
<i>Prot. vulgaris</i>	2000	0	0	0	0
<i>B. typhosus</i>	2000	0	0	0	0
<i>B. Para-Ty. B.</i>	2000	0	0	0	0
<i>B. aerogenes</i>	1000				0
<i>B. fecal. alk.</i>	1000				0
<i>B. tularensis</i>	1000				0

It will be noted that the staphylococci resist the action of urea better than other species tested, especially *Staphylococcus aureus*, which survived after twenty-four hours of exposure. *Staphylococcus albus* survived for four hours of exposure, but not after twenty-four hours. One strain of *Streptococcus fecalis* was viable after twenty-four hours, but growth was not obtained at the earlier periods. The last species were tested only after twenty-four hours of exposure.

The results so far obtained suggest that urea may be of considerable value in the treatment of purulent discharges of many types and in the treatment, also, of suppurating wounds producing foul odors. This latter use of urea has been reported recently by Millar.<sup>5</sup> His paper has received rather severe criticism only because it has been generally believed that urea has no bactericidal power. The cheapness and harmlessness of urea should encourage other investigations of its clinical use.

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# PERIPHERAL NEURITIS AND ABORTION FOLLOWING DINITROPHENOL THERAPY\*

## REPORT OF A CASE

ERVIN EPSTEIN, M.D., AND HAROLD ROSENBLUM, M.D., SAN FRANCISCO, CALIF.

**A**LPHA-DINITROPHENOL ( $C_6H_3(NO_2)_2OH$ ) was introduced into medical therapy by Cutting, Mehrtens, and Tainter<sup>1</sup> for the treatment of obesity. This compound was of clinical importance during the World War, however, since numerous instances of dinitrophenol poisoning were reported among munition factory workers at this time. The purpose of this report is to present an instance of peripheral neuritis and abortion occurring in an obese woman following the administration of dinitrophenol.

## REPORT OF CASE

Mrs. A. S. is a white, married, housewife aged thirty-four years. She is the mother of three normal children and had had no abortions or miscarriages. The remainder of her past history and family history were irrelevant.

The patient was treated in the Metabolic Clinic of the Mount Zion Hospital for obesity. On Feb. 20, 1934, treatment for this condition was instituted with an initial daily dose of 100 mg. of sodium dinitrophenol. This is the equivalent of approximately 75 mg. of the acid 1-2-4 dinitrophenol. The daily dose was increased to 200 mg. of sodium dinitrophenol daily on March 6, 1934, and further increased to 300 mg. one week later. There was no further change in the dosage until April 3, 1934, when it was increased to 400 mg. and then to 500 mg. one week later. On this therapy she lost 21.5 kilos (47½ pounds) from 107.0 kilos (235½ pounds) to 85.5 kilos (188¼ pounds) in twenty-six weeks. Her basal metabolic rate before treatment was plus 6 per cent and reached a maximum of plus 88 per cent after the administration of 500 mg. of sodium dinitrophenol a day regularly for five weeks, the test being made one and one-half hours after the morning dose of 200 mg.

On June 12, 1934, the patient reported that for the previous two weeks she had noted a severe burning sensation in the balls of both feet and in all of the toes. Sharp, shooting pains were also experienced occasionally in these regions. Examination in the Neurological Clinic on July 3, 1934, by Dr. W. F. Beerman showed "a low stocking analgesia and anesthesia of the left foot extending from the external condyle downward including the whole of the foot. The toes were quite red. There was also hypalgesia to pin-prick on all of the toes and on the entire plantar surface of the right foot."

On July 7, 1934, she stated that her menstrual period was two weeks overdue. Her periods had previously been regular. A pelvic examination revealed that the uterus had the size, shape, and consistency of a six-week gestation. An Aschheim-Zondek Test on the urine on Aug. 7, 1934, was reported as being positive.

The dinitrophenol was discontinued on July 24, 1934, but was started again on Aug. 7, 1934. During the period in which she was not taking the drug, there was no marked improvement in the condition of her feet.

The patient entered Mount Zion Hospital on Aug. 22, 1934, having again been on dinitrophenol therapy for two weeks. At this time she said that she had been perfectly well, except for the burning sensations and pains in her feet, until the day before entry. There

\*From the Department of Medicine and Department of Obstetrics, Mount Zion Hospital.  
Received for publication, February 12, 1935.

had been no symptoms of pregnancy other than the cessation of menses. At 11:40 A.M. on Aug. 21, 1934, she had helped lift a very heavy woman. At about 6:00 P.M. of the same day she noted a slight amount of vaginal bleeding and this was somewhat increased the next morning. However, she continued to take the regular doses of dinitrophenol during this time. At about noon she began to bleed profusely from the vagina and was still bleeding when she was brought into the hospital. Her underclothes were found to be soaked with blood. Examination showed her skin to be pale, cold, and clammy. The oral temperature was 36.4° C. (97.5° F.). The heart sounds were faint and the pulse was rapid and almost imperceptible. She was very short of breath and fainted several times before treatment could be instituted. The patient also complained of abdominal and precordial pains. Treatment was given for shock and hemorrhage and one hour after entry the uterus was completely emptied by means of a dilatation and curettage. No fetus was found. After the shock and hemorrhage had been controlled, the patient complained of feeling "hot." Dinitrophenol therapy was discontinued as soon as the patient entered the hospital and has not been started again.

The patient admitted that she did not wish to have this baby but on numerous occasions stated consistently that neither she nor anyone else had done anything to terminate the pregnancy.

Physical examination on Aug. 23, 1934, revealed an obese female of about thirty-five years of age. The temperature was 37.5° C. (100° F.), pulse 106 per minute, and respirations 24 per minute. The skin was warm and moist and both the skin and mucous membranes were pale. No palpable lymph nodes were found. Fairly marked hypertrichosis of the chin was present. The skull, eyes, ears, nose, mouth, and neck were not remarkable. The tonsils were present and slightly enlarged. The breasts were pendulous, the nipples were inverted and there was no increase in the pigmentation of the areolae. The lungs were clear and the heart was normal. The blood pressure was 100 systolic and 60 diastolic. The peripheral arteries were soft. The radial pulses were moderately weak, but were otherwise not remarkable. The abdomen was negative except that it was flabby and exhibited numerous striae. The abdominal reflexes were not obtained, probably because of the obese and atonic abdominal wall. Inspection of the external genitalia showed no evidence of continued hemorrhage.

Examination of the extremities revealed no abnormalities in the arms. The biceps and triceps reflexes were active and equal. Examination of the lower extremities showed analgesia of the soles of the feet and of the distal phalanges of all the toes. There was hypesthesia over the remainder of the dorsal aspect of the feet extending up along the inner side of both calves. The vibratory sense was absent at the ankles and knees but normal over all bony prominences above the pelvis. The deep muscle sense was entirely lost in the toes. The muscle power and coordination were normal. The patellar and achilles reflexes were very difficult to obtain but were present and equal. The plantar reflexes were normal and there was no clonus present.

*Laboratory Findings.*—The blood count on entry showed hemoglobin 79 per cent (Sahli), or 12.3 gm. per 100 c.c. of blood; red blood cells 4,320,000 per c.mm.; white blood cells 17,100 per c.mm. The differential count showed polymorphonuclears 74 per cent, eosinophiles 4 per cent, monocytes 5 per cent, and lymphocytes 17 per cent. Eighty-nine per cent of the polymorphonuclear leucocytes were filamented. The Wassermann and Kahn reactions on the blood serum were negative. On entry, the blood gave a positive test for alpha-dinitrophenol according to the method of Bolliger.<sup>2</sup>

The urine was negative except for a one-plus albumin reaction.

The spinal fluid on Aug. 24, 1934, showed no cells, a negative globulin test and a colloidal gold reaction of 0000000000. A test for alpha-dinitrophenol was negative.

Microscopic examination of the curettings from the uterus showed decidual reaction of the endometrium with formation of chorionic villi indicating pregnancy.

The basal metabolic rate on Aug. 26, 1934, was plus 1 per cent.

*Subsequent History.*—The patient was next seen in the Out-Patient Department on Sept. 6, 1934. At this time, although she still complained of some numbness in her feet, neurologic examination indicated a marked improvement as evidenced by the disappearance of most of the abnormal findings noted on Aug. 23, 1934.

Slight diminution to tactile and painful sensations over the soles of the feet were the only residual abnormal findings. This was two weeks after dinitrophenol had been discontinued. One week later she was normal both subjectively and objectively. There has been no return of symptoms to date.

#### TOXICITY OF ALPHA-DINITROPHENOL

Dinitrophenol may produce toxic effects as a result of overdosage or by doses within the normal therapeutic range in hypersensitive patients. Mayer (quoted by Perkins<sup>3</sup>) cites the fact that in munition factories, toxic manifestations develop in only a small number of workers despite the fact that others are working under identical conditions. He also states that chronic rheumatism, chronic alcoholism, tuberculosis, renal disorders, and hepatic disease decrease the tolerance of the patient to dinitrophenol.

Matzger<sup>4</sup> reported 157 patients on whom he performed skin tests with dinitrophenol. All gave negative reactions, but three later developed skin manifestations while under dinitrophenol therapy. This suggests that these tests will not be useful in detecting patients who are likely to develop a hypersensitivity to this drug. Frumess<sup>5</sup> reported an instance of a patient with a skin eruption due to dinitrophenol who gave positive scratch and passive transfer tests with dinitrophenol.

A review of the available literature failed to reveal any reports of patients suffering from fully developed peripheral neuritis or abortion due to dinitrophenol. The toxic effects vary greatly in each patient. Among those noted are asthenia, loss of weight, backache, pain in the chest, hyperpyrexia, feeling of warmth, excessive perspiration, night sweats, acidosis, coma, and even death.\* It may also cause euphoria, mental confusion, headache, vertigo, and pains and paresthesias in the extremities. Its toxicity for the cardiovascular system may be manifested by tachycardia and fall in blood pressure. Dinitrophenol may cause pharyngitis and otitis media. The French have noted a type of shortness of breath which they termed "thermic dyspnea" as a complication of dinitrophenol administration. Vomiting also may be encountered. The effect on the liver may take the form of a toxic hepatitis with jaundice. Among the numerous possible skin complications may be mentioned urticaria, pruritic maculopapular erythema, hemorrhagic bullae, exfoliative dermatitis and jaundice. Dinitrophenol may also lead to agranulocytic angina. Hyperglycemia may also occur. Albuminuria has also been noted in patients taking dinitrophenol.

Several autopsies have been reported but there has been no uniformity of findings in these few cases.

#### DISCUSSION

The evidence presented strongly suggests that the peripheral neuritis in this patient was due to dinitrophenol. Numerous instances of patients suffering from pains and paresthesias in the extremities while on dinitrophenol medication have been reported.<sup>12, 13</sup> The neuritis developed in this patient while the drug was being taken and disappeared within three weeks after its administration was discontinued. Trinitrotoluene,<sup>14</sup> benzol<sup>15</sup> and other compounds chemically closely related to dinitrophenol have caused true peripheral neuritis. From this evi-

\*For references to toxicity see 1,2,5 to 14.

dence it would seem justifiable to conclude, at least tentatively, that the neuritis in this patient was due to the dinitrophenol.

Deacon<sup>17</sup> in 1918 stated that it is not particularly dangerous for pregnant women to work in munition factories. However, in her series of 101 women who became pregnant while working in a munition factory, thirteen had early miscarriages. She attempts to explain only four of these. This is a high percentage and suggests the possibility of the existence of an unrecognized toxic agent as a causative factor in these miscarriages. Unfortunately no statement is made as to whether or not dinitrophenol was used in this munition factory.

A miscarriage could be due to the drug passing through the placenta and causing the death of the fetus, or the mechanism might be analogous to the early premature separation of the placenta sometimes seen in hyperthyroidism.<sup>18</sup> The absence of any discoverable etiologic factor except dinitrophenol in our patient makes it probable that the drug was responsible for the abortion. The importance of these two complications makes it advisable that the possibility of dinitrophenol causing peripheral neuritis and miscarriage be brought before the medical profession.

#### SUMMARY

1. A case is reported in which peripheral neuritis and abortion occurred in a patient while under dinitrophenol therapy.

2. A brief review of the problem of dinitrophenol toxicity is presented. This includes evidence which indicates the probable relationship between the administration of dinitrophenol and the occurrence of peripheral neuritis and miscarriage in the case presented.

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# THE RESISTANCE OF RED BLOOD CELLS TO HEMOLYSIS IN HYPOTONIC SOLUTIONS OF SODIUM CHLORIDE\*

## OBSERVATIONS IN BLOOD DISORDERS

GENEVA A. DALAND, S.B., AND KATHARINE WORTHLEY, A.B., BOSTON, MASS.

### INTRODUCTION

THE osmotic resistance of the red blood cells has been of interest since 1883, when Hamburger<sup>1</sup> first developed a method of determining the susceptibility to hemolysis of the red blood cells in hypotonic concentrations of salt solution. Partly because there have been many modifications of technique employed by different investigators and partly because the observations have not always been made upon comparable stages of the same disease, inconstant results of the test in different disorders, particularly in anemia, have caused its value to be debated. The purpose of this investigation was, therefore, to study by means of a refined technique the resistance of the red blood cells to hypotonic salt solution in different disorders, especially in the same individual over a prolonged period of time. Furthermore, it was desired to discover if any relationship existed between resistance and the physical and chemical constitution of the red cells.

### METHODS

*Determination of the Resistance of Red Blood Cells.*—One hundred cubic centimeters of stock salt solution were made from chemically pure sodium chloride for each 0.02 per cent concentration between 0.80 and 0.04 per cent inclusive, and kept in tightly stoppered bottles. One cubic centimeter of each concentration of sodium chloride was put into each of a series of appropriately labeled test tubes (10 mm. by 75 mm.). The tubes had previously been thoroughly cleaned without the use of acid.

Five cubic centimeters of venous blood were rendered incoagulable for the blood counts and resistance studies, using 0.05 c.c. of a 20 per cent solution of potassium oxalate. The cells of 2 c.c. of this sample were washed twice by adding 5 c.c. of 0.85 per cent sodium chloride to the whole blood, centrifuging twice at 1,500 revolutions per minute for fifteen minutes, and removing the supernatant fluid. The total volume was made up with 0.85 per cent sodium chloride to five times the volume of packed cells. One-tenth of a cubic centimeter of this 20 per cent cell suspension was added to each tube of salt solution and thoroughly mixed. The tubes were then allowed to stand from one to three hours in the refrigerator. Then all the tubes were packed into carriers and centrifuged for fifteen minutes at 1,500 revolutions per minute. The tubes were then arranged in order in a rack, and the points of hemolysis observed. Thus

\*From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard Medical School.  
Received for publication, December 1, 1934.

the technical variations were minimized by employing uniformly measured volumes of cells and sodium chloride solution, and by observing the end-points with particular care as described below.

*Minimum Resistance.*—*Trace* of hemolysis was determined by comparing the supernatant fluid in each tube in question with a control tube against a source of light with a blue daylight glass. The slightest color in the supernatant fluid was considered to indicate a *trace* of hemolysis. *Definite* hemolysis was determined by noting the highest concentration of sodium chloride in which an obvious reddish color appeared in the supernatant fluid.

*Maximum Resistance.*—*Partial* hemolysis was characterized by the persistence of a definite globule of cells or "button" in the bottom of the tube. The point of *complete* hemolysis was determined microscopically by observing the sediment in each tube below the "button." Hemolysis was considered complete when less than forty unhemolyzed red cells were present per field using a 40 mm. objective. The microscopic method of determining the end-point is of special value when the white blood count is high because leucocytes are much more resistant to hypotonic salt solution than are erythrocytes and, therefore, confuse the macroscopic observation of the end-point.

#### DETERMINATION OF HEMATOLOGIC DATA

Blood counts were done on the venous blood, using U. S. Bureau of Standards pipettes and counting chamber. The Sahli hemometer was standardized so that 100 per cent was equivalent to 21 volumes per cent of oxygen, assumed to be the capacity of 15.6 gm. of hemoglobin per 100 c.c. of blood. The Wintrobe hematocrit was used and indices determined.<sup>2</sup> The percentage of reticulocytes was determined by counting 1,000 red blood cells in smears of blood which had been brought in contact with brilliant cresyl blue in the moist state, fixed and stained with Wright's stain. The cell diameter measurements were made on fixed smears, using a projection apparatus according to the method of Price-Jones,<sup>3</sup> and the mean diameter and standard deviation calculated on 250 cells for each blood.

#### RESULTS

*Resistance to Hemolysis in Hypotonic Solutions of Sodium Chloride of the Red Blood Cells in Blood Disorders.*—More than 1,000 observations of red cell resistance have been made on some 200 individuals. A summary of some of these observations is presented in Table I. An average of the results for each group does not give an entirely correct picture because of the wide range of variation which has been observed in the same type of disease; therefore, the highest and lowest values (extremes) for each group, as well as the average, are given. In the cases of anemia the observations included in Table I were all made before any potent therapy was given.

*Acute Blood Loss.*—In the cases of acute blood loss, although the anemia was severe and some reticulocytes were present, the remaining red blood cells were practically normal and their resistance remained normal. The variation, as shown in Table I, was no greater than that in the normal individuals.

TABLE I  
RESISTANCE TO HYPOTONIC SODIUM CHLORIDE SOLUTIONS OF THE RED BLOOD CELLS OF NORMAL INDIVIDUALS AND OF PATIENTS WITH VARIOUS BLOOD DISORDERS

DIAGNOSIS	NUMBER OF CASES	MEAN CORPUSCULAR VOLUME (CUBIC MICRA)		MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (PER CENT)		PERCENTAGE CONCENTRATION OF SODIUM CHLORIDE IN WHICH HEMOLYSIS OCCURRED											
						MINIMUM RESISTANCE				DEFINITE				PARTIAL			
						TRACE		EX- TREMES		AVER- AGE		EX- TREMES		AVER- AGE		EX- TREMES	
						EX- TREMES	AVER- AGE	EX- TREMES	AVER- AGE	EX- TREMES	AVER- AGE	EX- TREMES	AVER- AGE	EX- TREMES	AVER- AGE	EX- TREMES	AVER- AGE
Normal	20	97-81	91.6	35-29	31.8	35-29	0.48-0.46	0.48-0.44	0.46	0.46-0.40	0.44	0.46-0.40	0.44	0.36-0.30	0.33	0.30-0.24	0.27
Acute blood loss	5	111-92	101.9	35-33	33.4	35-33	0.48-0.44	0.46	0.46	0.44-0.40	0.42	0.44-0.40	0.42	0.34-0.28	0.30	0.28-0.24	0.26
Hypochromic anemia	17	76-51	65.4	29-20	25.4	29-20	0.60-0.48	0.52	0.52	0.52-0.42	0.50	0.52-0.42	0.50	0.32-0.18	0.28	0.26-0.10	0.19
Idiopathic hypochromic anemia with achlorhydria	2	80-57	68.5	28-24	26.2	28-24	0.54-0.48	0.51	0.51	0.52-0.46	0.49	0.52-0.46	0.49	0.24-0.18	0.21	0.20-0.16	0.18
Chronic blood loss	12	95-62	76.5	31-22	26.6	31-22	0.54-0.42	0.50	0.50	0.48-0.40	0.45	0.48-0.40	0.45	0.36-0.22	0.29	0.28-0.18	0.23
Poor diet and infection	3	83-73	78.5	27-25	26.1	27-25	0.54-0.48	0.50	0.50	0.50-0.44	0.47	0.50-0.44	0.47	0.30-0.24	0.26	0.23-0.16	0.18
Pregnancy	4	84-68	77.1	30-24	27.3	30-24	0.54-0.48	0.50	0.50	0.52-0.46	0.48	0.52-0.46	0.48	0.30-0.20	0.27	0.26-0.16	0.22
Thrombocytopenic purpura	3	94-73	83.6	34-24	29.2	34-24	0.52-0.46	0.48	0.48	0.50-0.40	0.44	0.50-0.40	0.44	0.31-0.28	0.31	0.26-0.18	0.23
Henophilia	2	84.4	84.4	30-3	30.3	30-3	0.52-0.46	0.46	0.46	0.46-0.40	0.42	0.46-0.40	0.42	0.26-0.16	0.20	0.26-0.18	0.27
Erythroblastic anemia (Cooley's)	5	117-68	92.4	33-23	27.7	33-23	0.76-0.50	0.58	0.58	0.60-0.46	0.49	0.60-0.46	0.49	0.26-0.16	0.20	0.18-0.08	0.13
Sickle cell anemia	1	93.1	93.1	30.4	30.4	30.4	0.60-0.42	0.49	0.49	0.50-0.38	0.44	0.50-0.38	0.44	0.36-0.26	0.32	0.30-0.24	0.27
Pernicious anemia	20	153-91	118.5	34-25	30.8	34-25	0.60-0.42	0.49	0.49	0.50-0.38	0.44	0.50-0.38	0.44	0.36-0.26	0.32	0.30-0.24	0.27
Macrocytic anemia of pregnancy	4	107-88	101.7	34-22	27.4	34-22	0.54-0.48	0.51	0.51	0.50-0.44	0.47	0.50-0.44	0.47	0.38-0.36	0.37	0.34-0.26	0.29
Osteosclerotic anemia	1	101.1	101.1	29.4	29.4	29.4	0.48-0.46	0.47	0.47	0.44-0.42	0.42	0.44-0.42	0.42	0.36-0.34	0.35	0.30-0.26	0.28
Polycythemia rubra vera	2	126-91	108.6	34-27	31.0	34-27	0.60-0.46	0.51	0.51	0.50-0.42	0.46	0.50-0.42	0.46	0.36-0.28	0.32	0.30-0.20	0.26
Chronic hemolytic jaundice	5	115-59	88.8	38-22	32.6	38-22	0.60-0.46	0.51	0.51	0.50-0.42	0.46	0.50-0.42	0.46	0.36-0.28	0.32	0.30-0.20	0.26
Before splenectomy	13	104-70	88.2	38-30	34.5	38-30	0.85-0.50	0.76	0.76	0.80-0.48	0.62	0.80-0.48	0.62	0.44-0.24	0.38	0.42-0.18	0.28
After splenectomy	3	96-81	86.8	33-25	33.5	33-25	0.70-0.64	0.66	0.66	0.62-0.60	0.60	0.62-0.60	0.60	0.36-0.34	0.35	0.30-0.26	0.29
Chronic lymphatic leucemia	3	107-89	96.0	32-30	31.2	32-30	0.52-0.48	0.50	0.50	0.50-0.46	0.48	0.50-0.46	0.48	0.36-0.34	0.35	0.30-0.26	0.30
Chronic myelogenous leucemia	7	103-77	92.0	34-27	30.6	34-27	0.80-0.50	0.58	0.58	0.60-0.46	0.53	0.60-0.46	0.53	0.36-0.24	0.33	0.32-0.22	0.28

*Hypochromic Anemia.*—The blood picture of hypochromic anemia is characterized by small cells poorly supplied with hemoglobin, showing considerable variation in size and shape, whether associated with bad diet, gastric anaecidity, blood loss or pregnancy. An increased span of resistance, especially with increased maximum resistance, has been observed by Minot,<sup>4</sup> Waugh,<sup>5</sup> and Scotti-Douglas and Dondi.<sup>6</sup> The association of increased resistance with microcytosis has been demonstrated by Scotti-Douglas and Dondi. Graham<sup>7</sup> emphasized the fact that the diminished minimum resistance in these cases is similar to that found in hemolytic jaundice and, on that basis, suggested that there is a relationship between the two diseases. He did not observe an increased maximum resistance.

The most characteristic alteration of red cell resistance in the group of thirty-eight patients with hypochromic anemia (recorded in Table I) was the increased maximum resistance of the cells. A decreased minimum resistance also occurred in some cases. The first group included seventeen women with idiopathic hypochromic anemia with achlorhydria. In ten of these complete hemolysis occurred in 0.20 per cent sodium chloride solution or below. Five of the patients had the syndrome of hypochromic anemia, dysphagia, and glossitis. In contrast to the report of Graham<sup>7</sup> the maximum resistance was increased in nearly all, which is contrary to the usual finding in chronic hemolytic jaundice.

In chronic blood loss, whether due to menorrhagia, hemorrhoids, carcinoma, thrombocytopenic purpura or hemophilia, the characteristic increased maximum resistance was found when the blood values were such as obtain in other types of hypochromic anemia. In four cases of hypochromic anemia associated with pregnancy and three cases with poor diet and infection, the resistance of the red cells was similar to that of other types of hypochromic anemia and apparently varied only with the severity of the anemia. On the whole, the span of resistance was not so great in these groups of cases as in those which had achlorhydria.

*Erythroblastic Anemia (Cooley's).*—In erythroblastic anemia which has been described recently by Cooley and Lee<sup>8</sup> and by Baty, Blackfan, and Diamond<sup>9</sup> there is an increased maximum resistance of the red blood cells, but there is a difference of opinion in regard to alterations in the minimum resistance. Observations of the resistance of the red blood cells in five patients (Table I) showed that the span of resistance was much greater in all than in normal individuals. The minimum resistance was decreased in three cases but the most striking change occurred in the maximum resistance. Complete hemolysis occurred in 0.14, 0.18, 0.10, 0.16, and 0.08 per cent sodium chloride solution, respectively. The increased maximum resistance thus resembles that found in severe hypochromic anemia. Observations on one case of sickle-cell anemia showed a similar increased maximum resistance.

In erythroblastic anemia, a type of hypochromic anemia probably not related to iron deficiency, the red cells show marked achromia with extreme variation in size and shape. A special feature of this type of blood is the presence of poikilocytes. The mean diameter of the cells was normal, below or above normal, but the deviation of the diameters from the mean and the coefficient of variation were high (standard deviation = 1.20 and 1.19, and coefficient of variation

= 17.10 per cent and 18.42 per cent). The marked increase in maximum resistance occurred in the same bloods which showed a great many small cells and poikilocytes which remained unhemolyzed in the dilute solutions.

*Pernicious Anemia.*—Although the resistance of the red blood cells in pernicious anemia in relapse varies much more than in normal individuals, the average values for maximum and minimum resistance have been reported<sup>10, 11, 12</sup> as very close to that of normal individuals. In twenty cases studied before treatment the abnormal feature was found in the minimum resistance. The trace of hemolysis either did not appear at all until there was color enough to be seen at a glance, and thus coincide with the point for definite hemolysis, or there was slight coloring of the supernatant fluid in several of the tubes above the point of definite hemolysis. This trace of hemolysis, due no doubt to the destruction of a few red cells, has often been overlooked or disregarded. The test is not of diagnostic value for pernicious anemia, because of the normal span of resistance in many cases and the wide range of variation. The resistance of the red cells of four patients with the pernicious (macrocytic) anemia of pregnancy gave similar results to those found in Addisonian pernicious anemia.

*Osteosclerotic Anemia.*—Occasional observations were made on a patient with osteosclerotic anemia recently reported by Chapman.<sup>13</sup> The patient had between 1 and 2 million red blood cells per cubic millimeter for more than a year. The resistance of the red cells was studied about two months after each of several transfusions. The minimum resistance was decreased throughout. The mean corpuscular volume was slightly above normal most of the time, while the mean corpuscular hemoglobin concentration was below normal. There were 5 to 10 per cent of reticulocytes present throughout this period.

*Aplastic Anemia.*—Increased minimum and a normal or decreased maximum resistance have been recorded as a feature of advanced aplastic anemia.<sup>14, 15, 16</sup> The consistent change has been stated to be a decreased maximum resistance and increased minimum resistance, producing a narrow span of resistance. Observations in the late stages of the disease of two patients showed a slightly decreased span of resistance.

*Polycythemia Rubra Vera.*—The resistance of the red cells in polycythemia rubra vera has been discussed by Minot and Buckman.<sup>17</sup> The variability reported by them was confirmed by the present study of five patients. In two patients the minimum resistance was 0.56 and 0.60 per cent sodium chloride, respectively, while the maximum resistance was practically normal. In other patients the minimum resistance was normal and the maximum resistance was increased, or a normal resistance was present. These differences may be manifestations of the varied red cell values which obtained in these patients.

*Chronic Hemolytic Jaundice.*—A study of thirteen cases of chronic hemolytic jaundice confirmed the observations of previous authors.<sup>18, 19, 20, 21</sup> A decrease in the minimum resistance was found in all these cases. The maximum resistance was decreased in eleven of the thirteen cases. The abnormal resistance of the red blood cells to hypotonic sodium chloride solution was not found to be an index of the severity of the anemia or of the clinical condition of the patient.

Observations on three patients with hemolytic jaundice were made one, two, and five years, respectively, after splenectomy. These patients were symptomatically well and the blood counts were normal, but the resistance, both minimum and maximum, was still decreased. This agrees with the work of other investigators that the resistance to hypotonic salt solution remains abnormal after splenectomy, although the number of reticulocytes becomes normal, icterus disappears and the clinical condition of the patient becomes greatly improved.

*Leucemia.*—Three cases of *chronic lymphatic leucemia* showed practically a normal span of resistance. At times during eight months of observation in one case the minimum resistance was decreased to 0.52 and 0.50 and the maximum resistance varied from 0.26 to 0.34 per cent of sodium chloride at different times. Complete hemolysis was very abrupt, as confirmed by direct microscopic examinations of the sediment. The resistance of the red cells in *chronic myelogenous leucemia* varied within wide limits. In the seven patients studied the minimum resistance was decreased. There seemed to be no definite correlation in these cases between the resistance span and the number of the red cells, or the number and age of the white cells.

*Progressive Changes in the Resistance of the Red Blood Cells of Patients During Treatment.*—Progressive changes in the resistance to hemolysis of the red cells were observed in certain types of anemia during a period of weeks or months while treatment was being given. As the degree of anemia changed there were changes in many of the corpuscular values; for example, in the mean corpuscular volume and hemoglobin content, and in the resistance to hemolysis. The consideration of the stage of the disease, therefore, is important in studying the resistance of the red blood cells.

*Hypochromic Anemia.*—In hypochromic anemia the abnormal resistance of the red cells approached normal values as the small cell volume and lowered hemoglobin concentration increased toward normal as the result of effective therapy. These characteristic changes are illustrated by observations upon three patients with hypochromic anemia made during the period of response to therapy with iron and are presented graphically in Fig. 1. The first case (Case 1) was a patient who had idiopathic hypochromic anemia with glossitis, dysphagia, and achlorhydria. The red blood cell count on admission was 1,900,000 per c.mm. and the hemoglobin was 19 per cent. The maximum resistance was increased, complete hemolysis occurring in as low a concentration as 0.10 per cent sodium chloride. The minimum resistance was only slightly diminished. The volume of the red blood cells was below normal (75 c. micra) and the mean corpuscular hemoglobin concentration was low (20.1 per cent). As the mean corpuscular volume and hemoglobin concentration rose in response to the daily administration of iron and ammonium citrate, the point of maximum resistance or complete hemolysis approached the normal value. The red cells also increased in thickness, the percentage of poikilocytes in the blood smears of this patient was 14.6 per cent, but four months later, when the red cell count, hemoglobin value, and resistance were nearer normal, this percentage was 6.6. The size of the

cells in a given sample also varied markedly. The standard deviation of the diameters of the cells and the coefficient of variation have been determined to show the heterogeneity of the cell population (Table II). This standard deviation is a measure of the dispersion of the diameters, their range in size, and the way in which the numerical frequencies of the diameters are arranged.<sup>3</sup> These values are very much increased in these severe hypochromic anemias, but return toward normal, showing greater uniformity of the cells coincident with the return of the resistance values to normal. Direct observations of the nonhemolyzed erythrocytes in the tubes of salt solution near the point of complete hemolysis indicated that the change from partial hemolysis (the tube in which the "button" of cells appears) to complete hemolysis was more gradual than that which occurred in the blood of normal individuals. When the sediments in the tubes

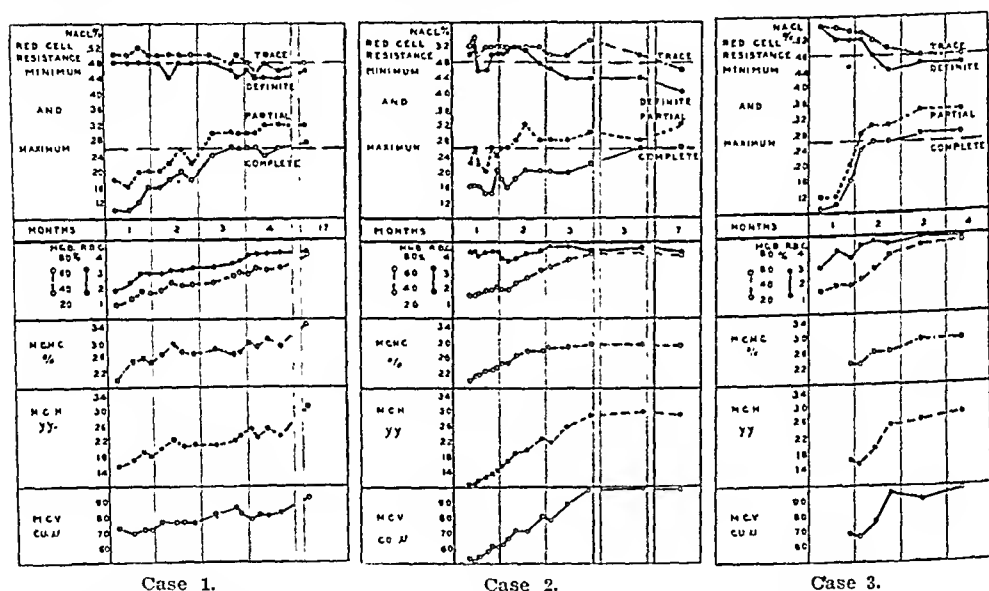


Fig. 1\*.—Resistance to hemolysis in hypotonic solutions of sodium chloride of the red blood cells in hypochromic anemia.

\*In Figures 1 to 4 inclusive the following abbreviations are used:

R.B.C., red blood cells in millions per c. mm.

Hgb., hemoglobin expressed in per cent; 100 per cent=15.6 gm.

M.C.H.C., mean corpuscular hemoglobin concentration in per cent.

M.C.H., mean corpuscular hemoglobin in micromicrograms ( $\gamma\gamma$ )

M.C.V., mean corpuscular volume in cubic micra (cu.  $\mu$ )

M.C.D., mean corpuscular diameter in micra ( $\mu$ )

M.C.T., mean corpuscular thickness in micra ( $\mu$ )

Red Cell.

Resistance, The average normal value for minimum resistance (trace of hemolysis) and maximum resistance (complete hemolysis) is shown by dash lines on each chart.

of salt solution of low concentration were observed under the microscope, polkiloeytes and small cells represented a large percentage of the nonhemolyzed cells.

In the second case of idiopathic hypochromic anemia the initial red count was 4,350,000 per c.mm. and the hemoglobin, 31 per cent, which represents a very low mean corpuscular hemoglobin. The maximum resistance was in-

TABLE II

CHANGES DURING TREATMENT IN THE SIZE OF THE CELLS AND RESISTANCE TO HYPOTONIC SODIUM CHLORIDE SOLUTIONS OF THE RED BLOOD CELLS IN TWO PATIENTS WITH HYPOCHROMIC ANEMIA

	R. B. C. (MILLIONS)	HEMOGLOBIN (PER CENT)	RETICULO- CYTES (PER CENT)	M. C. V. (C. MICRA)	M. C. D. (MICRA)	STANDARD DEVIATION OF DIAMETER	COEFFICIENT OF VARIATION (PER CENT)	M. C. T. (MICRA)	PERCENTAGE CONCENTRATION OF SODIUM CHLORIDE IN WHICH HEMOLYSIS OCCURRED			
									MINIMUM RESISTANCE		MAXIMUM RESISTANCE	
									TRACE	DEFINITE	PARTIAL	COMPLETE
Case 1												
1/29/32	2.9	35	0.9	73.2	7.44	1.08	14.5	1.67	0.50	0.48	0.20	0.12
2/12/32	3.2	46	0.5	76.3	7.31	1.06	14.5	1.81	0.50	0.44	0.22	0.18
3/21/32	3.7	55	1.0	86.0	7.42	0.86	11.59	1.99	0.48	0.46	0.30	0.26
4/ 6/32	4.1	66	1.2	82.1	7.52	0.75	9.96	1.84	0.44	0.44	0.30	0.26
4/22/32	4.1	65		83.0	7.24	0.64	8.83	2.02	0.46	0.44	0.32	0.26
Case 2												
7/13/32	4.3	31	1.6	51.7	6.33	0.83	13.1	1.64	0.54	0.52	0.26	0.16
7/21/32	4.4	36	1.2	57.1	6.68	0.89	13.32	1.62	0.52	0.46	0.20	0.14
8/10/32	3.8	46	12.2	70.6	7.65	0.97	12.67	1.54	0.52	0.52	0.28	0.18
8/17/32	4.2	53		70.5	7.67	1.12	14.6	1.53	0.52	0.52	0.32	0.20
10/23/34	4.5	91		93.8	7.60	0.65	8.55	2.06	0.48	0.44	0.34	0.30



creased to 0.16 per cent sodium chloride, returning to normal with the return of the red cell and hemoglobin values to normal. The minimum resistance was slightly decreased during the first three months of therapy and then returned to normal. During this period the mean corpuscular volume, which was unusually low (53 c. miera), doubled and the mean corpuscular hemoglobin returned to normal. The standard deviation of the red cell diameters from the mean and the coefficient of variation on this patient are also shown in Table II. These values showed some variation during the reticulocyte response but became lower, showing a decrease in the heterogeneity of the cells, as the span of resistance and other red cell values returned to normal.

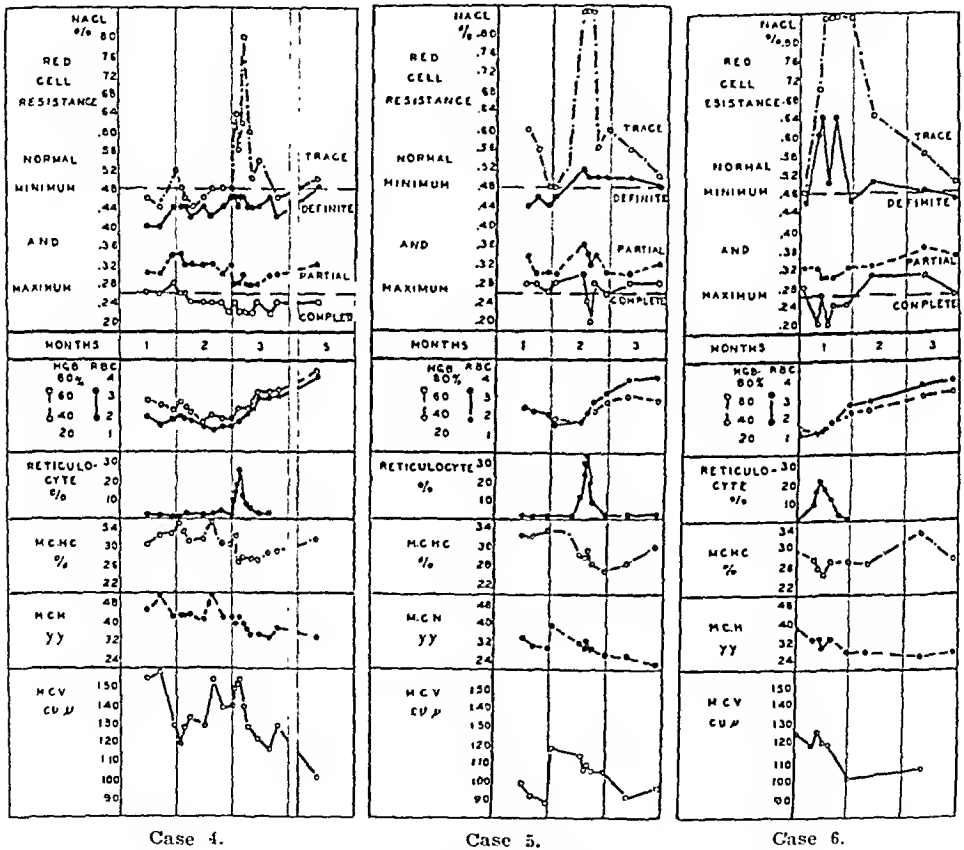


Fig. 2.—Resistance to hemolysis in hypotonic solutions of sodium chloride of the red blood cells in pernicious anemia.

The third patient presented in Fig. 1 had the microcytic hypochromic anemia of pregnancy and was first observed following delivery. Here the return of the resistance span to normal values was much more abrupt than in the other cases because of the rapid rise of blood values due to intensive iron therapy. During the first month following delivery the maximum resistance was increased markedly, as in the other cases. The minimum resistance was decreased during the first two months and returned to normal when the hemoglobin concentration had reached 30 per cent. The mean corpuscular volume

rose from 65 cubic micra to over 90 cubic micra coincident with the return of the maximum resistance to normal.

*Pernicious Anemia.*—Observations made upon three patients with pernicious anemia are presented in graphic form in Fig. 2 and serve to illustrate the typical phenomena in relation to changes in resistance occurring in liver-induced remissions in this disease. Since the original abnormality of the resistance in this condition is not so striking as in hypochromic anemia, the progressive changes occurring with improvement are not so striking as are those associated with the reticulocyte responses. Both minimum and maximum resistance may be altered at the time of increased production of young red cells. Thus, in each of these patients the reticulocyte response to effective liver therapy was accompanied by a decreased minimum resistance. Not only was the point of trace of hemolysis altered at the time of the reticulocyte response, but also the point of definite hemolysis. The occurrence of definite hemolysis in such high concentrations means that a great many of the cells became less resistant. Co-

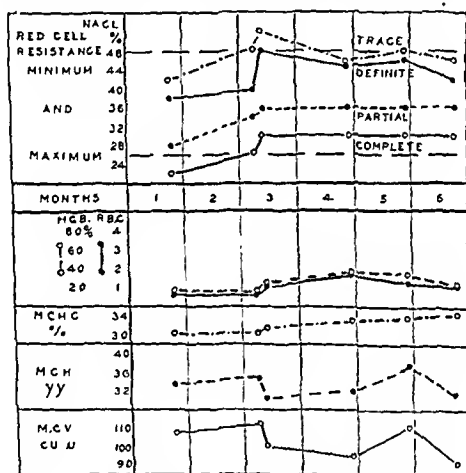


Fig. 3.—Case 7. Resistance to hemolysis in hypotonic solutions of sodium chloride of the red blood cells in aplastic anemia.

incident with the reticulocyte response and the decrease in the minimum resistance there was a sudden decrease in mean corpuscular hemoglobin and hemoglobin concentration. Some increase in the mean corpuscular volume also occurred. Since the new young cells (reticulocytes) are usually larger than those normally present in the blood stream<sup>23, 24, 25</sup> but relatively poor in hemoglobin, it is suggested that such cells may be responsible for the changes observed during the response to liver extract therapy. The minimum resistance was, however, not always decreased at the time of the reticulocyte response. Under these circumstances it was found that the mean concentration of hemoglobin was normal. After the red cells had reached at least a level of 4,000,000 per c.mm., the maximum and minimum resistance were normal.

The maximum resistance was practically normal in all three patients before treatment. There were marked increases in the maximum resistance at the time of the reticulocyte responses. A slight increase in the maximum resistance

developed in Case 4 during and after treatment. Coincident with this increased maximum resistance there was a decreased concentration of hemoglobin in the cells. Thus these cells became low in hemoglobin like the cells in the less severe cases of hypochromic anemia in which comparable changes in the maximum resistance occurred.

*Aplastic Anemia.*—The observations on two patients with aplastic anemia seem to show correlations between the normal or decreased span of resistance and the relative uniformity of the red cells. The red blood cells of both patients remained below 2,500,000 per c.mm. throughout the period of observation. The data from observations upon one of these cases are shown in Fig. 3. The span of resistance was decreased in four out of six observations. It may be seen

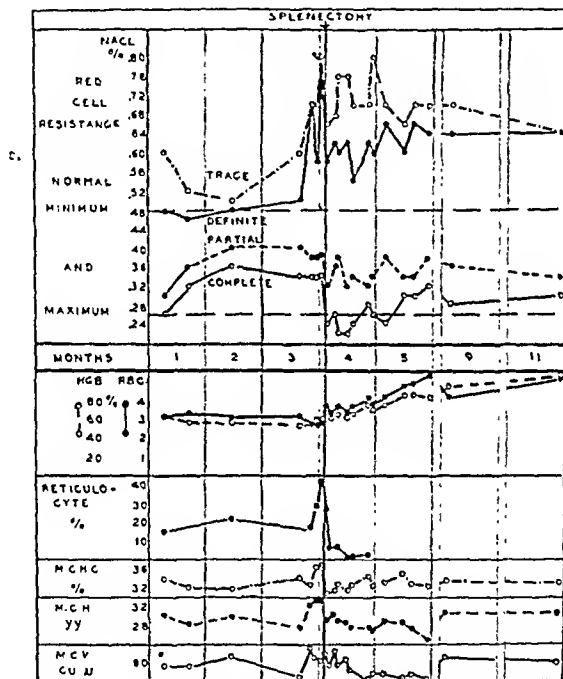


Fig. 4.—Case 8. Resistance to hemolysis in hypotonic solutions of sodium chloride of the red blood cells in chronic hemolytic jaundice.

that the difference between definite and partial hemolysis is very much less than normal in this case. The mean corpuscular hemoglobin concentration in this case was about normal, while the mean corpuscular volume and the mean corpuscular hemoglobin were greater than normal. The second case was similar in that the span of resistance was decreased in nine out of fourteen observations.

*Chronic Hemolytic Jaundice.*—Certain changes in resistance in hemolytic jaundice were observed in following the course of the individual cases. In Fig. 4 are presented data from a patient with congenital hemolytic jaundice who was studied for more than a year, during which time splenectomy was performed. Before splenectomy the minimum resistance varied from 0.50 to 0.80 per cent sodium chloride, and the maximum resistance, from 0.26 to 0.36 per

cent sodium chloride. Thus the span of resistance was less than normal in some observations. The hemoglobin content and volume of the red blood cells varied comparatively little during these three months until the last week before splenectomy. At this time a hemolytic crisis occurred and the reticulocytes increased from about 20 to 43 per cent, and the minimum resistance decreased to 0.80 per cent sodium chloride. The reticulocytes returned to normal almost immediately after splenectomy but the minimum resistance remained abnormal—a fact which has been observed by other investigators.<sup>19, 20, 21</sup> The markedly decreased minimum resistance fluctuated between 0.80 and 0.66 per cent sodium chloride during the first month after splenectomy, and at the end of seven months was still decreased to as much as 0.64 per cent sodium chloride. The maximum resistance is said to be increased after splenectomy, but, in this case, it remained increased only while the hemoglobin concentration was below normal. Following splenectomy there was a sudden relative decrease in the mean corpuscular hemoglobin concentration and gradual decrease in the mean corpuscular volume accompanied by an abnormally increased maximum resistance which persisted for nearly a month. Here, perhaps, is a resemblance to the conditions found in hypochromic anemia.

#### DISCUSSION

The greatest difficulty in determining from these observations what characteristics of the erythrocytes produce a given change in resistance is that the cell population is extremely heterogeneous in most pathologic conditions. To compare the end-points of a series of observations upon resistance with the mean values for cell volume and hemoglobin concentration is obviously not satisfactory. The breadth of the span of resistance was suggested by Chauffard<sup>18</sup> to have a relationship to the range of variation in the cell population. In order to demonstrate this variation a study of the standard deviation of the diameter in different types of cases has been made. It is not intended to imply that variation in diameter is the factor directly responsible for variation in resistance. Such a measurement is merely a practicable means of expressing *heterogeneity* of the cell population. In respect to resistance there may be some factor of heterogeneity of cell population less easy to measure. The standard deviation is a measure of the dispersion of the diameters, their range in size, and the way in which the numerical frequencies of the diameters are arranged. These figures have been determined for about thirty cases, including normal individuals, patients with pernicious anemia, hypochromic anemia, Cooley's anemia, hemolytic jaundice, acute blood loss, and aplastic anemia. In general, the greater the dispersion of the cell diameters, the greater is the span of resistance. The results on cases of hemolytic jaundice and pernicious anemia at the reticulocyte response seem to be exceptions. The progressive narrowing of the span of resistance in hypochromic anemia during treatment follows the same law. Examination of the blood smears of the cases which had a wide span of resistance also showed considerable variation in the amount of hemoglobin in different cells. The fact that a widened span of resistance in hypochromic anemia was often associated with increased maximum resistance and a more gradual appearance of the end-point is probably also an expression of greater heterogeneity. On

the other hand, in aplastic anemia the narrowed span of resistance may well be associated with the greater conformity to a given cell type, as was shown by the standard deviation of the diameters.

In 1922, Gänsslen<sup>25</sup> stated his belief that the cells in chronic hemolytic jaundice are more susceptible to hemolysis because they are more spherical than normal erythrocytes. According to Haden<sup>26</sup> this principle applies to other pathologic conditions in man, especially hypochromic anemia. In the blood of hypochromic anemia the presence of numerous poikilocytes (nonspherical cells) in the sediments in the lower concentrations of salt beyond the point of "button" is in accord with this principle. It is probable that the low hemoglobin concentration is an additional factor working for increased maximum resistance, since if the cells are not well filled with hemoglobin, the thickness of the cells might conceivably be decreased.

Although a lowered mean corpuscular volume and mean corpuscular hemoglobin concentration are also correlated with an increased maximum resistance in hypochromic anemia, Cooley's anemia, and polycythemia, and although the normal maximum resistance in pernicious anemia and aplastic anemia is associated with more normal values for mean corpuscular volume and hemoglobin concentration, it is probable that these correlations appear because of their influence on cell shape.

Reticulated, as well as nonreticulated, cells show physical changes which may be related to the changes in the resistance. By some investigators the increased maximum resistance is associated with increased reticulocytes, while by others the decreased minimum resistance is correlated with the increased reticulocytes. Simmel<sup>27</sup> gave a comprehensive review of the literature and concluded that difference in age of cells may cause this variation in resistance. Minot and Buckman<sup>17</sup> associated the increased span of resistance in erythremia with different ages of red cells in the blood. Either the minimum or the maximum resistance or both may be altered with the increase of reticulocytes, but there is no definite correlation between the actual number of reticulocytes in the blood and either the minimum or maximum resistance, if all types of cases are studied as one group. A study of the diameters of the cells in fixed preparations showed that the reticulocytes in different conditions or stages of the same conditions vary markedly in size from reticulated macrocytes to reticulated microcytes, and that the amount of hemoglobin in these cells also varied considerably. The best explanation that can be given at this time is that the sudden physical changes of the red cells in pernicious anemia at the time of the reticulocyte response, which are demonstrably associated with increase of the mean corpuscular volume or lowering of the mean corpuscular hemoglobin concentration, may change the resistance of the erythrocytes. These alterations in resistance are apparently not due to any quality inherent in the age of the red blood cells but to changes in their size, shape, hemoglobin concentration, or other factors.

#### SUMMARY

1. A method has been described for determining the resistance of the red blood cells to hypotonic salt solution. The amount of solution and the amount of

red cell suspension are kept uniform throughout. The careful reading of the end-points makes it possible to observe slight variations in the resistance of the red cells.

2. The usual observations of the maximum and minimum resistance in the various blood disorders may be summarized as follows:

*Normal maximum resistance* occurs in pernicious anemia and lymphatic leucemia.

*Increased maximum resistance* may be found in various sorts of hypochromic anemia, erythroblastic anemia (Cooley's), polycythemia rubra vera, and in myelogenous leucemia, depending upon the degree of anemia present. In idiopathic thrombocytopenic purpura and hemophilia the changes are apparently consistent with the degree of hypochromic anemia. After splenectomy in hemolytic jaundice and occasionally in pernicious anemia during treatment, a temporary hypochromic blood picture may be accompanied by increased maximum resistance.

*Decreased maximum resistance* is usually found in hemolytic jaundice and in advanced aplastic anemia.

*Normal minimum resistance* may be found in moderate degrees of hypochromic anemia, including that of purpura, hemophilia, Banti's disease, sometimes in pernicious anemia and in polycythemia vera. Rarely it is normal in hemolytic jaundice.

*Increased minimum resistance* occurs in pernicious anemia in severe relapse and in aplastic anemia.

*Decreased minimum resistance* is usually found in hemolytic jaundice and may be found in hypochromic anemia, erythroblastic anemia, polycythemia vera, and myelogenous leucemia.

3. In general, with the exception of hemolytic jaundice and pernicious anemia in remission, the standard deviation of the cell diameters increases as the span of resistance increases. This variation in cell diameter is somewhat representative of the heterogeneity of the cell population and may be better correlated with variations in resistance than mean values. In hypochromic anemia, as the standard deviation of cell diameters from the mean decreases as a result of therapy, there is a progressive decrease in the span of resistance.

4. The increased maximum resistance in hypochromic anemia, Cooley's anemia, and polycythemia rubra vera is associated with a lowered mean corpuscular volume and mean corpuscular hemoglobin concentration. The latter may be important in causing flattening of the erythrocytes observed by Haden<sup>26</sup> in this condition. According to the theory of Gänsslen<sup>25</sup> the numerous poikilocytes should be an additional factor making for increased resistance, since they present striking deviations from the spherical form associated with a decreased resistance in hemolytic jaundice.

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## A CORRELATION OF ANATOMICAL FINDINGS WITH ABNORMALITIES OF THE $Q_3$ -WAVE\*

FREDERIC FELDMAN, B.S., M.D., AND DANIEL KORNBLUM, M.D.,  
BROOKLYN, N. Y.

CONSIDERABLE attention has been directed recently to disturbances of the initial part of the ventricular complex in the electrocardiogram, particularly a deep  $Q$ -wave in Lead III. Such a wave is a normal feature in cardiographs exhibiting right axis deviation. However, a great deal of discussion has been aroused as to its possible pathologic significance in cases with normal axis or left axis deviation. Parkinson and Bedford,<sup>1</sup> and Levine and Brown<sup>2</sup> reported the finding of a deep  $Q$ -wave in Lead III of the electrocardiogram in cases of coronary occlusion. Pardee<sup>3</sup> and Willius<sup>4</sup> found a deep  $Q$ -wave in Lead III in a variety of clinical conditions, notably anginal syndrome, arterial hypertension, arteriosclerosis with myocardial fibrosis, and pregnancy.

From their anatomical studies, and for theoretical reasons discussed in their paper, Fenichel and Kugell<sup>5</sup> concluded that injury to the smaller subdivisions of the conducting mechanism in the posterior part of the septum is probably responsible for the development of the characteristic  $Q$ -wave. Other authors,<sup>6-8</sup> on the other hand, believe that a deep  $Q$ -wave in Lead III can often be explained by a change in the axis of the heart. Wilson, et al.,<sup>9</sup> and later Barnes<sup>10</sup> have recently called attention to the frequency of a deep  $Q_3$ -wave in records showing inversion of  $T_3$  in cases of infarction of the basal portion of the posterior wall of the left ventricle. Such records were designated  $Q_3T_3$  types and were considered by Barnes to be more definitely correlated with basal ventricular infarction than  $T_3$  changes alone. Since the posterior portion of the septum is frequently involved in cases of infarction of the left ventricle posteriorly, the question arises whether the deep  $Q_3$  is correlated (1) solely with the septal pathology, as stated by Fenichel and Kugell or (2) with the ventricular wall lesion, which may or may not involve the septum posteriorly. Wilson, et al., and later Barnes further noted the presence of deep  $Q_1$ -waves in association with  $T_1$  inversion ( $Q_1T_1$  types) in cases of anterior wall infarction, also involving, as a rule, the anterior portion of the septum.

With the hope of throwing some light on this subject, an attempt is made in this report to correlate abnormalities of the  $Q$ -wave and inversion of the  $T$ -wave with pathologic findings in the heart, in a series of autopsies that have come under our observation during a period of about two years.

\*From the Department of Pathology, Jewish Hospital of Brooklyn, Dr. Max Lederer, Director.

Received for publication, February 23, 1935.



Pardee's criteria for an abnormal Q-wave were as follows: An initial negative wave in Lead III of more than 25 per cent of the maximum deflection in any lead, immediately followed by a definite upright wave. In the present study, Pardee's criteria were followed except that those cases were also included which did not show a definite upward deflection immediately following the initial negative wave in Lead III (Fig. 2). Thus, cases with left axis deviation without an upward deflection at the beginning of the ventricular complex were considered abnormal by us. As has been pointed out by Shookhoff and Douglass, such an abnormal  $Q_3$ -wave does not correspond in time with  $Q_1$  or  $Q_2$  but is synchronous with parts of  $R_1$  or  $R_2$ . This is to be expected since the wave in question is very frequently a Q-wave combined with the main



Fig. 1.—Heart (Case 1815) showing old infarctions of posterior ventricular and posterior septal walls. A, Anterior aspect. P, Posterior aspect. P. V. W., Posterior ventricular wall with area of marked thinning and scarring. S, Extension of scarred area to posterior portion of septum.

downward deflection. We hesitate to add a new term to the nomenclature, and, therefore, we have designated the combined deflection as a deep  $Q_3$ -wave or as an abnormal Q-wave.

Fenichel and Kugell described two cases with L.V.P. in which the initial upward deflection in Lead III was less than 5 per cent of the maximum total deflection. Both of these cases showed pathologic changes similar to those in which they found a deep Q-wave, and they called this particular type of excursion an indeterminate Q-wave. This type of wave was encountered once in our study.

Of thirty-two pathologic hearts with recent electrocardiograms available (taken within a month of death), we discarded five cases with bundle-branch

TABLE I  
CASES WITHOUT OLD OR RECENT MYOCARDIAL INFARCTION

CASE	AUTOPSY NO.	PATHOLOGIC FINDINGS			ELECTROCARDIOGRAPHIC FINDINGS	
		CORONARY ARTERIES	VENTRICULAR WALLS	SEPTUM	INVERSION OF T-WAVE	ABNORMALITIES OF INITIAL COMPLEX
1	1598	Left coronary slightly sclerotic	Microscopic congestion and diffuse fibrosis	Microscopic congestion and fibrosis	None	None
2	1629	Arteritis both coronaries	Interstitial myocarditis	None	T <sub>3</sub>	None
3	1650	Normal	Microscopic fibrosis at apex	None	None	None
4	1671	Both coronary arteries sclerosed but patent	Diffuse microscopic fibrosis (sclerosis of aorta)	Diffuse microscopic fibrosis	None	None
5	1679	Both extremely sclerotic lumen constricted	Diffuse myocarditis	Diffuse myocarditis	T <sub>1</sub>	None
6	1692	Both sclerosed, patent	Intramural fibrosis (syphilis of aorta and aortic valve)	None	None	None
7	1700	Patent	Fibrinous pericarditis (terminal nephritis)	None	T <sub>1</sub> (T <sub>2</sub> isoelectric)	None
8	1738	Both thickened but patent	Hypertrophy. Hyaline changes	Hyaline changes	T <sub>1</sub>	None
9	1609	Normal	Carcinomatosis of pericardium	None	T <sub>1</sub> (T <sub>2</sub> diphasic)	None
10	1600	Normal	Rheumatic pancarditis; diffuse myocarditis	None	T <sub>1</sub>	None
11	1649	Normal.	Pericarditis, endocarditis, diffuse interstitial myocarditis	None	T <sub>2</sub> , T <sub>3</sub>	None
12	1672	Normal	Rheumatic pancarditis	Endocardial roughening	T <sub>1</sub>	None
13	1914	Normal	Chronic fibrous pericarditis	None	None	None
14	1691	Normal	Bacterial endocarditis. Diffuse suppurative myocarditis	Diffuse suppurative myocarditis	T <sub>3</sub>	None

TABLE II  
CASES OF OLD OR RECENT INFARCTION

CASE NO.	AU-TOPSY NO.	CORONARY ARTERY PATHOLOGY	PATHOLOGY OF VENTRICULAR WALLS	REGION OF VENTRICULAR WALLS INVOLVED	SEPTAL PATHOLOGY	ELECTROCARDIOGRAPHIC FINDINGS		
						IN-VERTED T-WAVE	ABNORMALITY OF INITIAL COMPLEX	AXIS
1	1660	Old thrombosis right coronary, left circumflex and left descending	Infarction, myomalacia and fibrosis anteriorly and posteriorly	Anterior and posterior	Infarction, myomalacia and fibrosis, anteriorly and posteriorly	None	Deep Q <sub>3</sub>	L. A. D.
2	1792	Recent occlusion right coronary. Left anterior descending a fibrous cord. Left circumflex narrowed	Area of recent infarction posteriorly near base. Old infarct, fibrotic, anteriorly	Anterior and posterior	Necrosis and scarring posteriorly	None	Deep Q <sub>3</sub>	L. A. D.
3	1815	Occlusion right coronary, left circumflex thrombosed; left descending occluded	Infarction, fibrotic, posteriorly near base. Suppurative myocarditis (traumatic rupture of heart)	Posterior	Infarction upper part posterior	T <sub>3</sub>	Deep Q <sub>2</sub>	L. A. D.
4	2057	Right circumflex occluded by organized and calcified thrombus	Myomalacia posteriorly, involving left ventricles and extending into right	Posterior	Myomalacia posteriorly	T <sub>3</sub>	Deep Q <sub>2</sub>	L. A. D.
5	1683	Left descending thrombosed	Infarction anteriorly at apex	Anterior	Infarction of anterior third and of lower part of posterior septum	T <sub>1</sub> , T <sub>2</sub>	Deep Q <sub>1</sub>	L. A. D.
6	992	Left descending occluded	Infarction anteriorly at apex	Anterior	Infarction lower half of septum anteriorly and posteriorly; adherent thrombus	T <sub>1</sub>	Deep Q <sub>3</sub>	Normal axis
7	970	Both coronaries occluded at ostia; free below	Infarction, fibrosis, necrosis anteriorly and posteriorly. Syphilitic aortitis	Anterior and posterior	Necrosis of entire septum	None*	Deep Q <sub>3</sub> (not quite 25%) Transient	Normal axis
8	1685	Left circumflex occluded	Infarction posterior wall of left ventricle	Posterior	Infarction upper part of posterior septum	None	Upward deflection, 3%	L. A. D.
9	1625	Occlusion descending branch of left coronary	Infarction of apex	Anterior	Infarction of anterior third of septum. Posterior septum free	T <sub>1</sub>	None	L. A. D.

\*Two later cardiographs showed inversion of T<sub>1</sub> and T<sub>2</sub> with no abnormal Q-wave.

block and four with right axis deviation. The twenty-three hearts which remained for study were divided into two groups, those without coronary occlusions and myocardial infarction, and those with old or recent myocardial infarction.

Table I records the pathologic and electrocardiographic findings in the former group. None of these cases showed any gross pathologic lesions in the ventricular walls or in the septum. However, diffuse myocardial changes or interstitial fibrosis, not visible to the naked eye, occurred quite commonly, both in the ventricular walls and in the septum. A deep or abnormal Q-wave did not appear in any of the electrocardiograms in any lead, although inversion of the T-wave occurred nine times.

The second group consisted of nine cases with old or recent myocardial infarction (see Table II). The posterior part of the septum was involved in eight of the nine cases. In seven of these eight cases there were abnormalities of the Q-wave in Lead III. In the remaining case (No. 6), the pathology involved primarily the anterior ventricular wall and septum, and only a small portion of the posterior part of the septum was involved, by extension. Incidentally, this

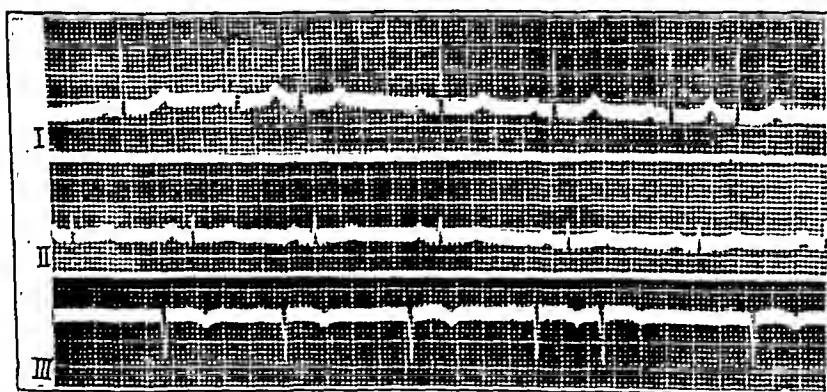


Fig. 2.—Electrocardiogram of the heart in Fig. 1, showing an abnormal Q-wave with inversion of the T-wave and an upward convexity of RT-segment in Lead III.

case showed a deep Q<sub>1</sub> in association with an inverted T<sub>1</sub>. Of the seven cases with abnormalities of Q<sub>3</sub>, five had definite deep Q<sub>3</sub>, one a transient prominent Q<sub>3</sub>, and one an initial upward deflection of less than 5 per cent, belonging to the type characterized by Fenichel and Kugell as the indeterminate Q<sub>3</sub>.

In three cases, infarction of the posterior ventricular wall was associated with the posterior septal lesions (Fig. 1). In two of these a T<sub>3</sub> inversion was present with a definite deep Q<sub>3</sub>. The third case had no inversion of T in any lead and showed an indeterminate type of Q-wave. In one case, although the infarction primarily involved the anterior ventricular wall and septum, it extended back sufficiently to involve the entire lower half of the posterior portion of the septum. This case had a T<sub>1</sub> inversion with the Q<sub>3</sub>. In three cases where infarctions occurred in both anterior and posterior portions of the myocardium, as well as in the posterior septal wall, no inversion of the T-wave occurred.

In summarizing, it may be said: That no case with a deep or abnormal Q-wave in Lead III failed to show gross posterior septal pathology; that none

of the cases free from gross lesions in the posterior part of the septum showed a deep Q-wave in Lead III. Seven of the eight cases (87.5 per cent) with posterior septal infarction had a deep or abnormal Q-wave in Lead III. In two of three cases of infarction of the posterior ventricular wall involving the posterior portion of the septum, an inverted  $T_2$  was associated with a deep  $Q_3$ ; also an inverted  $T_1$  was associated with a deep  $Q_3$  in one instance of anterior ventricular wall infarction extending to the posterior portion of the septum.

### CONCLUSIONS

1. A deep  $Q_3$  is a very frequent finding in cases of old or recent myocardial infarctions.
2. A deep  $Q_3$  appears to be correlated with pathology of the posterior part of the septum.
3. The  $Q_3T_3$  combination is suggestive of posterior ventricular infarction associated with posterior septal wall lesions; a  $Q_3T_1$  combination may occur when the posterior part of the septum is involved in the infarction of the anterior portion of the left ventricle and septum.

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## TOTAL SULPHUR OF TISSUE IN NORMAL AND ABNORMAL GROWTH (MOUSE CARCINOMA)\*

HERMAN BROWN, B.S., AND JOSEPH V. KLAUDER, M.D., PHILADELPHIA, PA.

CONSIDERABLE investigation has been conducted regarding the rôle that sulphydryl compounds play in cellular activity of normal and abnormal growth. It has been known for some time that cystine, the oxidized form of the sulphydryl-containing amino-acid cystine, is one of that limited group of amino acids which is indispensable for normal growth and development. Animals maintained on a cystine-poor diet show lowered vitality, retarded growth and defective hair.† In a previous communication<sup>7</sup> we reported studies and reviewed data showing the important rôle that cystine plays in the formation and development of keratin-containing tissue, especially hair and wool.‡

Whether reduced cystine alone is the primary agent in growth-promoting activity is controversial. Hammett is the chief proponent of this view. From the results of experiments with plant and animal material Hammett and his coworkers<sup>13, 14</sup> concluded that sulphydryl, as the substance occurring naturally in the cell, is the essential and universal stimulus to growth by increase in the number of cells, and that the reaction product, derivatives of sulphydryl are the natural inhibitors of this process so that the stimulation by sulphydryl is normally limited by the inhibitory action of the suboxidized forms of sulphydryl resulting from the normal oxidation of sulphydryl. Reimann and Hammett<sup>15</sup> have reported gross acceleration of wound healing by the simple external application not only of cystine but also of thioglucose and thio cresol.

Hammett's thesis that sulphydryl-containing substances are the essential and universal stimulus for normal growth in plants and animals is of considerable importance from the standpoint of the development and proliferative activity of malignant tumors.

From their studies of the action of sulphydryl on normal cell proliferation Hammett and Reimann formulated the following theory of the origin of cancer: The biologic basis of malignant tumors is the distortion of the sulphydryl equilibrium in the presence in the body of genetically determined lines

\*From the Research Institute of Cutaneous Medicine, Philadelphia.

Received for publication, December 15, 1934.

†Cystine is a unique amino acid since it appears that it cannot be synthesized by the animal organism. Gelling<sup>1</sup> showed that flowers of sulphur did not suffice to prevent loss of weight in rats on a poor cystine diet nor did inorganic sulphate in the study of Daniels and Rich.<sup>2</sup> Compounds closely related chemically to cystine such as *DL*-homocystine and silycolle acid<sup>3</sup> or taurine<sup>4</sup> were found inadequate to supplement . . . and thio- for maintenance or growth. Indeed, apparently *D*-cystine can . . . let either naturally occurring *L*-cystine in the animal economy.<sup>5</sup> Methionine, however, seems effectively to replace cystine in the diet.<sup>6</sup>

‡Animals maintained on a cystine-poor diet soon exhibit lowered vitality, retarded growth<sup>1</sup> and defective formation of tissues. The latter is especially apparent in keratin-containing tissue in which cystine content is relatively high.<sup>2</sup> These symptoms disappear and hair production of hair<sup>11</sup> and wool<sup>12</sup> is obtained. If the diet is rich in cystine an excessive

of cells in which the heightened nuclear reactivity of hypersensitivity to stimulation of proliferation by sulphhydryl characteristic of young, incompletely differentiated cells are retained, without resulting secondarily in increased differentiation. As the diverse architecture of malignant growths is essentially a consequence of the diverse anatomic and physiologic environments in which the proliferation of cells takes place, and as the increase in mitotic activity is in general followed by a decrease in the size of the cells, the rate of tumor growth is directly related to the interpretation of malignancy in terms of sulphhydryl calculated from cell size. Sarcomas and carcinomas represent different constitutional (connective tissue or epithelial) types. The difference in hydrogen ion concentration, carbohydrate metabolism, and proteolytic activity of the malignant cell in comparison with the normal cell are but sequelae of the heightened reproductivity of the malignant cell.

In a review and critical analysis of experimental work regarding the etiologic relation of glutathione and sulphhydryl to canceration, Huepner<sup>16</sup> concludes: "If one grants sulphhydryl substances a contributory part in the development of malignant growth it can be at best only a secondary rôle, remaining in the framework of their normal function."

Among data which Huepner reviewed, which he believed controverted an etiologic relation between sulphhydryl-containing substances and malignancy was the findings of a number of investigators that showed that the glutathione content of malignant tumors was not greater than that present in normal tissue.

Besides cysteine, thioncine and glutathione, thiolactic acid, thioglycollic acid and thioprotein may also play a rôle in promoting tissue growth and may all derive their sulphur from cystine in the diet.

In the studies here reported of the relation of sulphhydryl compounds to normal and abnormal growth we studied the total sulphur content of the skin of rabbits; and of different organs of mice maintained on normal and on cystine-poor diets; and noted the effect of such diet on the rate of growth of cancer in mice. An attempt was made to correlate the rate of growth with the sulphur content of the tumors. Total sulphur determination<sup>\*</sup> was selected in preference to the estimation of individual naturally occurring sulphhydryl compounds. Determination of total sulphur is more accurate and affords a composite picture of all the sulphhydryl compounds which may be operative in stimulating growth. Our purpose was not concerned with the relative effectiveness of reduced or oxidized forms of sulphhydryl substances but to correlate total sulphur content of tissue in normal and abnormal growth (carcinoma in mice).

The skins of rabbits were employed in our study of the total sulphur content of normal growing tissue. Skin is ideal for such study; it is a rapidly growing tissue; it is relatively high in sulphur;<sup>17</sup> it can be obtained free of blood and extraneous tissue; in normal growth it increases, proportionally, more rapidly in size than other organs. The skins of litter mates of rabbits

<sup>\*</sup>Sulphur was determined according to the method of Stockholm and Koch.<sup>17</sup>

were analyzed for total sulphur from birth to maturity. A series of such analyses were conducted and repeated on a second litter.

The results of this study shown in Table I are the averages of the two determinations for each age interval. It is to be observed that a definite decrease in total sulphur content occurred from birth to maturity. As the rapidity of growth decreased with the age of the animal the sulphur content likewise decreased.

In Thompson and Voegtlin's<sup>18</sup> study of the glutathione of the organs of the white rat it was observed that glutathione declined with the age of the animal. The decline was proportionately greater than that of the sulphur content of the skin of rabbits as shown in Table I.

TABLE I  
RELATION OF NORMAL GROWTH TO SULFUR CONTENT OF RABBIT SKIN

AGE	WEIGHT OF RABBIT GM.	SULPHUR IN SKIN % OF DRY WEIGHT
1 day	76	0.68
2 weeks	180	0.69
1 month	252	0.65
3 months	810	0.54
6 months	1200	0.44
8 months	1500	0.46

In order to determine the effect of a cystine-poor diet on the sulphur content of tissue, a number of white mice were placed on such a diet\* for five weeks at which time the sulphur content of muscle, liver, spleen, kidney, and skin of the animals was determined.† As a control study the same organs of white mice maintained on a normal diet were examined for total sulphur content.

The results of this study are shown in Table II. The figures for percentage sulphur represent averages of at least four analyses of pooled organs from

TABLE II  
SULPHUR ANALYSIS OF ORGANS OF NORMAL MICE AND MICE ON CYSTINE-POOR DIET\*

TISSUE	SULPHUR % OF DRY WEIGHT	
	NORMAL MICE	AFTER 5 WEEKS ON CYSTINE-POOR DIET
Muscle	0.49	0.39
Liver	0.59	0.45
Spleen	0.61	0.53
Kidney	0.62	0.44
Skin	0.50	0.41

\*Above results seem to indicate a slight but definite loss of body sulphur to meet the demands of metabolism due to withholding cystine from the diet. Mice were on this diet about five weeks.

several mice. It is to be observed in Table II that there is a loss of sulphur in the organs of the animals maintained on a cystine-poor diet. Fifty per

\*The diet, a modification of that proposed by Lightbody and Lewis<sup>19</sup> consisted of the following: Dried whole milk 17, Gelatin 3, dextrin 75, compressed yeast 1.5, cod liver oil 1.5, and Osborne and Mendel's salt mixture 2.

†Inorganic sulphur was of course included in the determination of total sulphur. The amount of inorganic sulphur in the organs examined is insignificant compared to total organic sulphur.



cent of the animals maintained for five weeks on a cystine-poor diet succumbed. It was, therefore, not practical to prolong the experiment. It is reasonable to assume that more striking results would have been obtained if the animals were maintained longer on the cystine-poor diet. However the results, in contrast to the control study are sufficiently definite to warrant the conclusion that a loss of body sulphur occurs following a cystine-poor diet.

In view of our observations that sulphur content of tissue decreased with the rate of normal growth, and, that loss of sulphur occurred after cystine-poor diet, we conducted the following study to determine the effect of cystine-poor diet on abnormal growth. Fifty white mice were placed on such a diet. At the end of four weeks twenty-two had died. The remaining twenty-eight were inoculated with an actively growing mouse carcinoma and the cystine-poor diet continued. As a control, twenty mice maintained on a normal diet were likewise inoculated. Both groups of mice were examined at weekly intervals for four consecutive weeks following the inoculation. The results of this study are shown in Table III. It is to be observed that the number of "takes" in the group of mice on the experimental diet were considerably less, and that the tumors grew more slowly than in the group on normal diet.

It is well known that under normal conditions of diet and health, some variation occurs in the rate of growth of mouse carcinoma. Generally, however, three weeks after inoculation the majority of mice present a tumor more than one-half the size of the animal. Occasionally, the tumor is smaller and may not attain the usual three-week size until eight weeks or longer. In some animals, too, the usual three-week size is not attained, the tumor stops growing, retrogresses and disappears. In the course of this study such slow growing tumors were observed among the mice in good health and on normal

TABLE III  
TUMOR GROWTH IN NORMAL MICE AND IN MICE ON CYSTINE-POOR DIET

NO. MICE INOCULATED	1 WK.	NO. OF "TAKES" AFTER		
		2 WK.	3 WK.	4 WK.
20 (controls)	10	16	18	18
*20 (cystine-poor diet)	0	2	6	7

\*Although twenty-eight on this diet were inoculated only twenty were alive at the end of the observation period.

diet. A comparative study of the sulphur content of slow growing, rapid growing tumors, and tumors in various stages of necrosis was made. This group included mice maintained on normal diet with one exception as noted in Table IV. The results as shown in this table are representative of other determinations not listed in the table. These results indicate that the sulphur content of mouse carcinoma correlates the rate of its growth. The more rapid its growth, the greater the sulphur content, the slower the growth, the less the sulphur content. The slowest growing tumor in our series had a sulphur content approximately that of normal muscle (mouse, see Table II) whereas, the sulphur content of the most rapidly growing tumor was more than 50 per

cent higher. The correlation of the sulphur content with the rate of growth applied to animals on normal diet, as well as to the animals on cystine-poor diet.

## DISCUSSION

Pertinent to this study is the fact that the organs that continue to grow after full body growth is attained contain a high amount of sulphur, for example, hair, nails, and skin; the more rapidly growing tissues contain more sulphur than other organs. It is not our purpose to endeavor to show that such growth receives its impetus from the sulphur compounds in these tissues; our results indicate, however, a coordination between sulphur content and rate of growth, normal and abnormal. Such coordination has been observed to exist between glutathione content of normal tissue and malignant growths.<sup>20</sup>

TABLE IV  
SULPHUR CONTENT OF TRANSPLANTED MOUSE CARCINOMA OF DIFFERENT  
RATES OF GROWTH

TUMOR	MOISTURE PER CENT	SULPHUR PER CENT DRY WEIGHT
Very slow growing. Not as large in 8 wk. as usual 3 wk. old tumor. <i>Normal diet</i>	81.4	0.47
Slow growing. Considerable necrosis. 6 wk. old. <i>Normal diet</i>	82.2	0.43
Very slow growing. Mouse on <i>Cystine-poor diet</i>	80.3	0.40
Normal growing tumor 3 wk. old. <i>Normal diet</i>	80.6	0.61
Normal growing tumor—slight central necrosis 4 wk. old. <i>Normal diet</i>	81.4	0.58
Normal growing tumor—considerable necrosis 5 wk. old. <i>Normal diet</i>	82.8	0.52
Very rapidly growing tumor. In 10 days had attained size equivalent to normal 3 wk. old tumors. <i>Normal diet</i>	80.8	0.74
Very rapidly growing tumor. In 10 days had attained size equivalent to normal 3 wk. old tumors. <i>Normal diet</i>	80.5	0.71
Very rapidly growing tumor. Necrotic. <i>Normal diet</i>	81.2	0.69

It is to be recalled that tumor growth in animals can be accelerated or retarded under different experimental conditions. Many investigators have held that vitamins A, B, C, and D stimulate the proliferation of transplantable neoplasms. Zondek<sup>21</sup> and his collaborators, and also Cannavo<sup>22</sup> found that subcutaneous injection of anterior hypophyseal hormone retarded the growth of transplanted Ehrlich carcinoma in mice. Frankel and Gereb<sup>23</sup> produced "takes" of Jensen rat sarcoma in mice maintained on a high vitamin diet whereas inoculation was not successful in the control animals on normal diet. Caspari and Ottensmeyer<sup>24</sup> were able to produce acceleration in the rate of growth of rat carcinoma through the administration of a diet rich in vitamin D.

The state of nutrition of the animal seems to be a factor in growth of mouse carcinoma. Hirata<sup>25</sup> studied the effect of different diets on the rate of growth of Flexner rat carcinoma. It was observed that growth of the tumor was retarded in animals on an inadequate diet, irrespective of the factors concerned in this inadequacy.

In studies published by the British Empire Cancer Campaign, it was found that any foodstuff, whose inclusion in the basal diet improved the general condition of the animals as shown by their appearance, increased life spans and rates of growth, also produced the same effect on the rates of growth of the benign and malignant tissues. Conversely, any food supplement which adversely affected the general condition of the animals decreased the rates of growth of the tumors.

Watson<sup>26</sup> observed an increased carcinogenic response in tar-treated mice when the diet on which they are maintained is supplemented with fresh liver. Such diet did not accelerate the onset of malignancy. There was no difference in the rate of development of malignancy if the administration of liver was discontinued after the first appearance of the benign warts.

The results of Hirokawa's<sup>27</sup> study are pertinent to our results herein reported. He fed different groups of mice rice dough, wheat bread, and "fu" (a kind of cracker made of gluten). These animals were implanted with mouse carcinoma. The results showed that the percentage of successful tumor inoculations and the rate of tumor growth in mice were distinctly diminished by feeding the animals with "fu" exclusively.

In studies herein reported animals maintained on a low cystine diet were in consequence of such diet in poor nutritional state. As mentioned in the foregoing the mortality rate was high. It is possible that the poor nutritional state of the animals played a rôle in retarding the rate of growth of the carcinoma irrespective of cystine insufficiency. However, since we correlated the rate of growth of transplanted tumors with the sulphur content and since we observed a loss of sulphur in organs of mice maintained on a cystine-poor diet, it is reasonable that cystine insufficiency played some rôle in retarding the rate of growth of the transplanted tumors in the animals maintained on a cystine-poor diet.

The occurrence of slow growing tumors in animals on a normal diet apparently cannot be attributed to cystine insufficiency. This observation, as well as foregoing considerations makes it apparent that other factors are operative in retarding the rate of growth of transplanted tumors. Regardless of these factors it appears from our studies that the rate of growth of transplanted tumors correlates the sulphur content.

#### SUMMARY AND CONCLUSIONS

Data are reviewed pertaining to the rôle that sulphydryl compounds play in cellular activity of normal and abnormal growth.

Study was made of the total sulphur content of tissue, in normal and in abnormal growth. The skin of rabbits was selected as representing normal

growing tissue. Analyses of total sulphur were made from birth to maturity. It was observed that total sulphur correlated the age of the animal, as rapidity of growth decreased with the age of the animal, the sulphur content likewise decreased.

The effect of cystine-poor diet on the sulphur content of muscle, liver, spleen, kidney, and skin of white mice was studied. There was a loss of sulphur in these organs in comparison with the control animals maintained on a normal diet. It appears from these studies that a loss of body sulphur occurs following a cystine-poor diet.

Total sulphur content of transplanted mouse carcinoma in animals on normal diet and in cystine-poor diet was made. It was observed that the rate of growth of the tumors correlated that sulphur content, the slower the growth the less the sulphur content, the more rapid the growth the greater the sulphur content. Data are reviewed pertaining to factors operative in retarding the rate of growth of transplanted tumors. It appears that regardless of factors involved in retarding the rate of growth of the tumors in the animals studied, the sulphur content correlated the rate of growth.

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2101 PINE STREET

1934 SPRUCE STREET

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## THE ETIOLOGIC RELATIONSHIP OF AMIDOPYRINE TO AGRANULOCYTOSIS\*

FRED STENN, M.D., CHICAGO, ILL.

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THE rôle that amidopyrine plays in agranulocytosis is not altogether clear. Since 1931, when Kraeke<sup>1</sup> suggested the possible relation between this disease and the ingestion of coal tar derivatives, many reports have appeared in this country, Scandinavia, and Holland, viewing the therapeutic use of amidopyrine with disfavor. Foremost in this regard have been those of Watkins,<sup>2</sup> Roberts and Kraehe,<sup>3</sup> Kraeke and Parker,<sup>4</sup> Madison and Squier,<sup>5</sup> Randall,<sup>6</sup> Hoffman, Butt, and Hiekey,<sup>7</sup> Holten, Nielsen and Transbol,<sup>8</sup> Seeman,<sup>9</sup> and Fitz-Hugh,<sup>10</sup> among others, each of whom presents cases giving a history of the taking of amidopyrine or related drugs. However, Jackson<sup>11</sup> doubts the significance of these drugs. In his study of twenty-seven cases of agranulocytosis only seven could be attributed, though with question, to the ingestion of amidopyrine.

At the present time there is no convincing experimental evidence that amidopyrine can produce agranulocytosis in laboratory animals, which fact does not necessarily mean that agranulocytosis in man may not be produced by this drug. Madison and Squier<sup>5</sup> reported that they had produced agranulocytosis in a single rabbit but failed in eighteen others, and this solitary positive result has not been repeated by the many others who have given amidopyrine to animals. Incomplete oxidation of the drugs containing the benzene ring (ace-

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\*From the Department of Pathology, University of Chicago.  
Received for publication, December 22, 1934.

taulid, phenacetine, amidopyrine and arsephenamine) leads, according to Kraeke,<sup>12</sup> to the formation of catechol, para-quinone, and quinone, which, he suggests, may be the responsible agents. In a personal communication Kraeke and Parker<sup>13</sup> informed me that they have obtained agranulocytosis in rabbits injected with quinone. (I have failed to obtain similar results in twenty rabbits that were injected daily for three weeks by the subcutaneous route with quinone in doses of 0.002 to 0.100 gm.) Miller<sup>14</sup> after administering orally to sixteen dogs 0.3 gm. per kilo of amidopyrine for four weeks found no marked decrease in the number of granulocytes in the peripheral blood. Suggestive, however, is the fact that histologic study of the bone marrow of the femur revealed a decrease or almost complete absence of granulocytes.

In an effort to investigate the effect of amidopyrine on the white and differential blood count I undertook a study of some 120 animals, comprising the guinea pig, the rabbit, and the monkey, over a period of eight months. Amidopyrine (Metz) was given orally to one group in doses from a fraction of a grain to three drams, and blood counts and differentials were taken daily. The symptoms were stupor, diarrhea, and loss of weight. Many of the animals died of pneumonia, some from amidopyrine poisoning, but at no time was agranulocytosis observed. This work was repeated with subcutaneous, intraperitoneal, and intravenous injections, but no change in the blood picture occurred. With another group an attempt was made to sensitize some of the animals treated, and these were given a one-month rest period, after which the medication was again given, still without effect. Incidentally, a pregnant guinea pig was injected with huge doses of the drug for one and a half months, but the newborn guinea pig and the mother both had normal blood pictures.

The next problem contended with was: Do severe anemia, damage to the bone marrow, starvation, chronic infection and toxemia, or cachexia, constitute the soil upon which agranulocytosis develops when amidopyrine is administered? The first group of animals in this series was bled daily until the red blood count fell between 1,500,000 to 2,000,000 and the red cells in the smear showed anisocytosis, poikilocytosis, and polychromasia. Despite this preparation the white blood count failed to fall upon prolonged administration of the drug. In the second group, small quantities of benzene in olive oil were injected subcutaneously until a severe neutropenia and anemia were produced. In most of the animals the white blood count fell to below 1,500. To one-half of this group amidopyrine was given orally and these animals showed, peculiarly enough, a more rapid rise in the white blood count and polymorphonuclear differential picture than in the other animals not given the drug. The animals of the third group were starved for five days and the drug administered, but without avail. In the fourth group animals were injected subcutaneously with broth cultures of *B. subtilis*, *Streptococcus viridans*, and *Salmonella suipestifer*, and one month later, with the infection still persisting, they were given heavy doses of amidopyrine orally during three weeks. The white blood counts were elevated during the period of amidopyrine treatment, and the differentials showed a predominance of polymorphonuclear leucocytes.

## SUMMARY

Administration of amidopyrine to guinea pigs, rabbits, and monkeys, both with and without preliminary anemia or bone marrow injury, failed to produce any appreciable granulocytopenia.

I am indebted to Mr. Maurice Kadin of Chicago and Miss Nettie Dash of Philadelphia for their technical assistance.

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## IMMUNITY PHENOMENA IN TUBERCULOSIS\*

SAMUEL A. LEVINSON, M.D., CHICAGO, ILL.

THE study of resistance to tuberculous infection embraces numerous biologic interactions. We must consider the cellular factors, the effect of sensitization, intercellular and intracellular digestion, humoral and fixed antibodies, endocrine and vitamin and chemical equilibriums. These factors become more difficult to interpret when pathologic complications, various constitutional differences, disturbances of the autonomic system, metabolic and emotional factors, and environmental influences and differences in the virus are added to the general changes that occur in patients.

The pathologic anatomists have demonstrated the peculiar liability of the slender individual, and clinicians have demonstrated the same fact in clinical studies and analytic analyses. This natural resistance is, according to Petersen,<sup>1</sup> not only genotypic, but may be influenced by such factors as menstruation, diet, season, and psychic status; but constitution, genotypic and paratypic, must be kept in mind as a determining factor in an infectious disease such as tuberculosis.

The human organism in attempting to overcome infection, as in tuberculous infection under discussion, attempts to overcome it in one of two ways, either by resorption or encapsulation. It is obvious that inhibition of digestion and suppression of inflammatory response is of great importance for the improvement of the patient with tuberculosis.

Together with Petersen, I<sup>2</sup> investigated some of the interrelations of resistance and the chemical status of a group of tuberculous patients (all men, 83 in number) in various stages of clinical activity. This investigation among other things included a study of the constitution, calcium-potassium ratio, cholesterol, tuberculin, and reaction to epinephrine. We wish to review briefly the latter findings in relationship to tuberculosis.

*Constitution.*—As far as the patient with pulmonary tuberculosis is concerned, increased inflammatory reactivity is usually associated with increased clinical activity. When the constitutional reactivity is lessened, the clinical course is relatively benign. Among the various lines of approach, particularly in the form of therapy, emphasis is directed toward specific immunity, with the attack directed toward the virus; chemotherapy with the emphasis against the destruction of the specific virus without injury to the cell; and last, the beneficial reactions of the tissue cells as a result of stimulation, with the accompanying suppression of the unfavorable ones.

\*From the Department of Medicine and Pathology, University of Illinois College of Medicine.

Received for publication, January 4, 1935.

<sup>1</sup>Presented before the Mississippi Valley Sanatorium Association, September 27, 1934, Cedar Rapids, Iowa.



The result of clinical and laboratory study has given us an opportunity of classifying patients into the sympathicotonic and vagotonic groups. It is obvious that the autonomic nervous system as a general part of the vegetative balance is involved. Epinephrine makes the capillary endothelium less permeable and is the representative agent for producing sympathetic effects. The "sympathicotonic" person has more permeable capillaries and the blood pressure may be low. The rest stage may be looked upon as the state of tissues with contracted vessels and less permeable capillaries. However, in some patients the injection of epinephrine hydrochloride lowers the blood pressure and increases the permeability. In the "vagotonic" person the blood pressure is higher, the metabolic rate is lowered, and the capillaries are less permeable. Stimulation of the so-called parasympathetic nerves may result in dilatation of the vessels and increased permeability which may mean tissue activity. In terms of clinical medicine the function of the capillaries may be expressed thus: Relatively impermeable capillaries are usually found associated with relatively chronic tuberculosis, and greater permeability with active tuberculosis.

The conclusion would be that increase in capillary permeability, however produced, unfavorably influences a tuberculous process.

*Calcium-Potassium Ratio.*—In a small series of cases studied by Voorhoeve,<sup>3</sup> he maintains that he noticed no striking difference in the calcium balance in tuberculous and nontuberculous subjects. Bonanno<sup>4</sup> in his study of 60 cases of pulmonary tuberculosis in various states reports no appreciable change in the calcium potassium content of the blood commensurate with the severity of the disease. A similar observation was made by Varela<sup>5</sup> who studied the total calcium of the serum and the fraction of ionized calcium in tuberculous patients and found that they remain within normal limits. Broekbank<sup>6</sup> observed that when his patients were graded according to severity, on the average the calcium was decreased in quantity in the serum when the disease was acute, and that it was increased when the disease was healed, with proportionate results in the intermediate stages. The difference amounted to about 20 per cent. The serum calcium was not diminished in patients who are coughing up blood, as compared with patients in a similar stage of the disease but without that symptom.

In an analysis of our 83 tuberculous patients we find that when this group is arranged according to clinical classification as minimal, moderately advanced, and far advanced, it is noticed that the minimal group has the highest value of potassium-calcium ratio and the more advanced lower values, but there is no progression or regularity. If the patients are classified according to the weight curve, there is a reduction of the calcium from the gaining to the losing group. When a similar study was made of these patients on the basis of prognosis, those who were gaining had the highest calcium levels. The significance of the calcium metabolism in our patients studied indicates that those who were gaining maintained a high calcium level as part of their picture of resistance. This occurs without loss from the body and the patients are said to be in "calcium balance." The group of patients who were losing were in a negative calcium balance, although the calcium level was high as it represents the calcium leaving the tissue and passing out from the body.

As far as potassium is concerned, we were unable to ascertain any clear-cut influence of the potassium levels on any clinical features studied. The potassium-calcium ratio has less influence on the tuberculous process than has the actual amount of calcium.

*Cholesterol.*—Clinical study has shown that tuberculous patients who have a high cholesterol content tend toward improvement, and conversely as the cholesterol content decreases the prognosis is grave. Gavrilu and Vior,<sup>7</sup> from their study of the cholesterol content of the blood serum from 100 patients, conclude that in the localized and diffuse fibrous forms of pulmonary tuberculosis there occurs a hypercholesteremia, particularly when the lesions are inactive and are not undergoing evolution. Hypocholesteremia is found in the diffuse ulcerocaseous tuberculosis when the lesions are active.

The cholesterol study made on our tuberculous patients substantiates the findings of Gavrilu and Vior in that the patients with good prognosis and inactive lesions had a higher cholesterol ratio than the patients with poor prognosis and active lesions. We have also noted that exudative pleurisies do not seem to influence materially the cholesterol content. In fact the highest cholesterol content was noted in some of our patients who had pleurisy.

Experimentally, Shope<sup>8</sup> has demonstrated that cholesterol administered intraperitoneally prolonged the lives of tuberculous guinea pigs when the infection was of an acute type produced by the injection of small doses of virulent human tuberculous organisms. When the infection was of a chronic type produced by the injection of small doses of human tuberculosis of a low virulence, the intraperitoneal injection of cholesterol did not prolong the lives of the guinea pigs.

Hinze<sup>9</sup> examined 69 patients with pulmonary tuberculosis and concludes that the greater the cholesterol content of the blood the greater the resistance of the patient to tuberculosis.

Palacio<sup>10</sup> attempts to prognosticate the outcome of pulmonary tuberculosis by cholesterol determinations and concludes that the quantity of cholesterol in the blood constitutes an index to the general condition of the patient.

There are several factors which modify the cholesterol content of the blood in tuberculous patients, namely, activity or inactivity of the lesions; state of immunity of the organisms; the pathologic anatomic form of the disease.

Gavrilu and Vior are of the opinion that hypercholesteremia indicates a state of a well-developed immunity and inactive pulmonary lesions, and is, therefore, of good prognostic import, while hypocholesteremia denotes a feeble state of immunity and acute lesions, and is, therefore, of bad prognostic import.

*Tuberculin Reaction.*—When making tuberculin skin tests, we must bear in mind the reactivity of the skin and the speed of the reaction before interpreting the results in terms of immunity. Thus the size of the reaction at the site of the injection of tuberculin, as measured after twenty-four hours, varied in our series from 0 to 28 mm. There is no difference in the clinical prognosis of our patients as determined by the tuberculin reaction. It was noted that those patients who gave a small skin reaction usually were patients with min-

imal clinical manifestation. The group with clinical manifestations of far-advanced tuberculosis gave the larger reactions.

The persistency of the tuberculin reaction was not in direct relation to the deaths. We observed that the cases in the far-advanced group showed a persistent and marked flare, and the gain in weight was associated with the largest reactions.

Ossoinig<sup>11</sup> observed at different times a peculiar increase and decrease of the tuberculin reaction occurring in the majority of the patients tested. He believes that climatic influences may play a rôle, but has not definitely been able to correlate them.

*Reaction to Epinephrine.*—We have divided the intracutaneous reaction to epinephrine from the observations made of the wheal and the flare. The average size of the wheal at the site of injection in our "normal" series was 19.2 mm. and in the tuberculous group it averaged 15.2 mm. More deaths occurred in our patients with small wheals, while in the group with large wheals, the prognosis seemed better, and more of the cases were at least stationary. The group with the most favorable prognosis seemed to be that in which the wheal was intermediate. Our study further indicates that the flare of the reaction to epinephrine and capillary permeability are somewhat greater in the group with large wheals; and a similar manifestation holds true for the reaction to tuberculin.

The average flare following the intracutaneous injection of epinephrine of "normal" men had a radius of 8.8 mm., while in the tuberculous patients it had a radius of 6.8 mm. The deaths in our series occurred in the group with the smaller flares. There appeared to be no significant relation of the size of the flare to the clinical classification, the prognosis, or the weight curve.

#### DISCUSSION

In order to correlate a number of biologic reactions that are observed in diseased conditions, it is necessary to know similar changes occurring in "normal" individuals. These observations are to be correlated with clinical symptoms and with resistance to an infectious disease like tuberculosis. It is important, therefore, to determine the alterations in the biologic tests employed in order to estimate increased or decreased resistance to tuberculosis.

In the previous report we<sup>12</sup> have shown that resistance to tuberculosis means complete walling off from general tissue activity, that is impermeability: anatomically, in the sense of a complete connective tissue encapsulation; and functionally, in the sense that there shall be lessened exchange of products between the focus and the normal tissues. Conversely, absorption of bacillary and tissue debris may lead to the dissemination of the virus.

Substances that stimulate and dilate capillaries, with incidental increase in permeability, must be potentially injurious. Substances that fatigue the capillary (with delay in its reversibility) must be harmful. Substances that make the capillary less permeable must be essentially beneficent.

When capillary endothelium is stimulated and becomes more permeable, calcium leaves the cell and the tissues become more acid. This is associated

clinically with exudative and progressive tuberculosis, with the increase in parasympathetic tonus (blood pressure lowered, low blood sugar, increased catabolism, etc.). With lessened permeability of the capillary wall the arteriole tonus is high, blood sugar (liver function) normal, tissues bind calcium, and the reaction is toward the alkaline side.

*Constitution.*—In view of the fact that constitution plays such an important rôle in resistance to infection, a brief review of this subject and its clinical applicability will be given. Von Versueher<sup>13</sup> is of the opinion that constitution is an organically fixed condition. Bauer<sup>14</sup> places emphasis on the importance of constitution in differentiating a group of biologically inferior persons, extreme variants from the normal, in adaptability, in vitality, and in resistance. For practical purposes for study, persons have been grouped as (1) normal (normotype), (2) slender (asthenic type with small heart), (3) heavy (pyknic type). In a similar fashion the French school associated the slender type with lessened resistance to pulmonary infections, and the heavy type with diabetes, arteriosclerosis and rheumatism. The following classification of the slender and broad types is taken from Petersen, "Constitution and Disease."<sup>15</sup>

## THE SLENDER TYPE

(Leptosomic, longitudinal, linear, dolichomorphic; asthenia as an extreme variant)

The individual is long

Extremities are long

The face is narrow, oval; the nasal bridge is narrow

The neck is long

The chest is long and flat, the costovertebral angle is small

The diaphragm is low

Dolichocephalic

Poor connective tissue reactivity

Bony framework is delicate

Skin is thin

Hair is fine, usually well preserved

The tissue storage is deficient (few reserves)

The heart is small (hypertrophy poor)

The arterial system is narrow

The superficial capillaries not marked

The viscera are mobile

Visceroptosis, varices, intussusception, prolapses and hernias common

Bodily circumference small

Smooth muscles hypotonic

## THE BROAD TYPE

(Plethoric, apoplectic, pyknic, gouty, "arthritis," the round type, brachymorphic)

The individual is broad

Extremities are short

The face is broad, round; the nasal bridge is broad

The neck is short

The chest is barrel-shaped, the costovertebral angle is larger, the intercostal space is narrower

The diaphragm is high

Brachycephalic

Pronounced connective tissue reactivity (fibrous)

Bony framework is strong

Skin is heavy and coarse

Hair is coarse and abundant, frequently lost early

The tissue storage is pronounced (reserves good, especially fat, glycogen, etc.)

The heart is large (hypertrophy good)

The arterial system is large

The superficial capillaries dilated

The viscera are well fixed

Rare

Bodily circumference large

Smooth muscles hypertonic

Sluggish general reactions except nervous and vasomotor systems	Hyperirritable in childhood; sluggish in later life
Thyroid activity may be increased	Thyroid activity lessened
Psychic classifications, schizothymic	Psychic manifestations, cyclothymic
Larynx large, voice barytone	Larynx small, voice tenor
Far sighted	Near sighted
Mechanical adaptation to overcome distance and time	Mechanical adaptation to overcome mass
Disease disposition, tuberculosis	Disease disposition, early exudative diathesis, late wear and tear diseases, metabolic diseases (gout, diabetes, concretions, sensitizations, etc.)
Moderate lymphatic reaction	Pronounced lymphatic reaction

When we attempt to interpret the results of our investigations on the basis of the above constitutional types, we notice that in the group of slender persons there is a low calcium-potassium ratio, an increased vascular reaction to epinephrine, lower cholesterol, and a lesser reaction of the skin to epinephrine. The slender person, in contrast to the heavy person, has an increased basal metabolic rate, a slightly lower  $\text{CO}_2$  combining power of the serum, higher resistance of the skin to electric current and a longer Kromayer light reaction time.

Aside from the constitutional types of persons, the age of the individual may probably be considered in reference to tuberculosis. Lydtin<sup>15</sup> examined in approximately 200 patients the relation of the age of the individual to the form of tuberculosis. The percentage of the proliferating, cirrhotic and exudative forms is the same in every age. The development of these forms is different. In younger persons the tendency goes from a proliferating to an exudative form, while the latter in older people has temporary ameliorations of the cirrhosis and cavity types.

Another phase in the constitutional types in reference to resistance is the question of the height of the individual. Frassetto<sup>16</sup> found that the average height of recent levies for the army is 2.5 cm. greater than that of the levies of 1859-1863, not only in healthy men but also in tuberculous patients. The height of patients with pulmonary tuberculosis exceeded by 2.5 cm. that of the healthy subjects, not only in recent but also in old levies. The chest girth in pulmonary patients is diminished by 2.6 cm. in the recent as compared with old levies, while in the healthy subjects, the reduction amounts to only 1.5 cm. In the old levies the chest girth of the tuberculous differed from that of the healthy subjects by 0.9 cm., and in the recent by 2 cm. The average weight of the tuberculous (aged twenty to fifty) in the recent levies is 12.5 kg. less than the weight corresponding to the average height of the tuberculous subjects, which was 167.5 cm.

Seasonal changes are known to influence the course of tuberculosis. Grönberg's<sup>17</sup> studies on 2,180 patients with pulmonary tuberculosis in Finland, comprising about 42,000 weighings and covering a period of seven years, show that the curve of the average monthly increase in body weight was practically

the same from January to April (245 to 262 gm.) dropping somewhat in May (232 gm.), rising rather sharply during the summer months to reach its peak in September (385 gm.), then sinking to the minimum in December (216 gm.). There was marked similarity between the weight curves and the curves of the average monthly temperature of the air and hours of sunshine. These observations on the relation between warmth and sunshine and body weight agree with results from sanitariums for the tuberculous in widely different parts of the world.

*Calcium.*—Zondek<sup>18</sup> calls attention to the fact that two balances are of greatest physiologic importance, the H ion concentration, and the potassium-calcium ratio. With cellular activity, the calcium concentration of the cell membrane is diminished. Calcium leaves the cell, and there is a relative increase in potassium in the cell. The cell is more permeable and the protein becomes hydrated (Zondek<sup>19</sup>). Thus, cellular activity can be measured by calcium and potassium concentrations. From the stage of activity that of rest is reached with an apparent increase of calcium at the cell surface, relative impermeability and dehydration. The concentration of either calcium or potassium in the serum may mirror the cellular change, but the serum levels do not change the reaction of the cells.

In reference to calcium metabolism it has been shown that it is closely associated with the clinical condition of the patient. We have observed, for example, that more deaths occurred in our patients who have had a low calcium level. Patients with healed tuberculosis have a higher calcium level, and those whose prognoses are good also have a higher calcium content. In contrast to this, we have observed in our study that patients with poor prognoses have a low calcium level. There apparently must exist some correlation between the calcium level and the activity of a tuberculous process, as there is some importance in the calcium in tuberculosis in regard to the reactivity of the tissue and its influence on the disease process. For example, Sternberg<sup>20</sup> found a lower serum calcium in most advanced cases while Schaeffer<sup>21</sup> has found increase in calcium in the lung in chronic tuberculosis. Hoyle<sup>22</sup> has noted some clinical improvement in patients with a higher calcium level; in fact, Hennes<sup>23</sup> noticed that animals exposed to calcium dust or other dust showed less tuberculosis than the control animals which were not exposed to dust. This fact has been applied therapeutically, and Siegel<sup>24</sup> has observed improvement in his patients who were treated by calcium inhalations.

*Cholesterol.*—It has been shown that cholesterol metabolism is diminished in infectious diseases and particularly with progressive tuberculosis. Shope has observed that as the cholesterol level is increased the prolongation of life in experimentally infected guinea pigs was noted. In fact, the relationship of cholesterol and other fats in the treatment of tuberculosis is an old empiric observation and is one of the factors that is still used today as a form of therapy in tuberculosis. We have observed that as a rule, in our investigations, low cholesterol in tuberculosis is associated with poor prognosis; a similar observation was noted by Reiter.<sup>25</sup>

*Skin Reactions.*—In reference to the reactivity of the skin in tuberculosis we have used the cantharides plaster applied to the skin.<sup>26</sup> This is a chemical irritant and acts primarily on the cell membranes. Specific sensitization is not employed in this test but the analogy to the tuberculin reaction is made here because of the skin irritation produced when a specific and nonspecific skin test is employed.

During the febrile stage there exists a peripheral vasoconstriction. The cantharides can act on the cell, but little or no exudate forms. There is direct injury to the cells. Applied to tuberculin and the tuberculin reaction, it means

TABLE I

COMPARISON OF CALCIUM, POTASSIUM, CHOLESTEROL, EPINEPHRINE, AND TUBERCULIN LEVELS IN PULMONARY TUBERCULOSIS

		MINIMAL	MODERATELY ADVANCED	FAR ADVANCED
Calcium		9.9	10.5	10.3
Normal	9.7			
Potassium		19.6	18.3	19.0
Normal	20.3			
Calcium Potassium Ratio		2.0	1.76	1.88
Normal	2.1			
Cholesterol		235.0	200.0	157.0
Normal	140-170			
Epinephrine				
Wheal		Medium wheal	Small wheal	Large wheal
Flare		Small flare	Medium flare	Large flare
Tuberculin				
End of twenty-four hours		Small wheal	Medium wheal	Large wheal
Persistent reaction		Small reaction	Medium reaction	Large reaction

that during the febrile stage of an intercurrent disease the tuberculin reaction is inhibited, because of the change of the status of the skin. During the terminal stage of tuberculosis this factor is also operative. During the stage when there is a peripheral vascular dilatation, when the cantharides plaster is applied, a strong local skin reaction results. Applied to the tuberculin reaction this corresponds to the period of maximum response when sensitization takes place at a time during which peripheral vasodilatation is compensating for the increased metabolism going on in the body. The autonomic skin function is practically normal as far as the lability of the skin is concerned. Then as the pathologic lesion in the lung, for example, becomes quiescent and the patient goes on to recovery, the cell membranes as in the skin are no longer irritated to the same degree by the same amount of irritant. In other words, the cell membranes have become less permeable. These reactions are wholly nonspecific effects which influence the reactivity of the skin in general, but they are of importance for the problem of tuberculosis, because the vegetative status of the skin and the vasomotor activity are underlying factors that determine the type of reaction to all toxic agents.

It is obvious, therefore, that in the intricate biologic picture of inflammation presented by tuberculosis, innumerable possibilities exist. We should emphasize that in clinical tuberculosis it is the vascular mechanism that must always be in the foreground of consideration.

## CONCLUSIONS

1. We have made biochemical studies on 83 patients with pulmonary tuberculosis (minimal to far advanced) and have here discussed briefly the subject of constitution, calcium, potassium, cholesterol, epinephrine skin tests, and tuberculin in relation to resistance to tuberculosis.

2. The results indicate that substances that stimulate and dilate capillaries, with incidental increase in permeability, must be potentially injurious to the host. Substances that make the capillary less permeable must be essentially beneficial to the organism.

3. As the clinical status in tuberculosis progresses (exudative and progressive tuberculosis), the capillary endothelium is stimulated and becomes more permeable, calcium leaves the cells, and the tissues become more acid (parasympathetic tonus, low blood sugar, increased catabolism, etc.).

4. When the organism is in a state of lessened permeability of the capillaries (sympathetic tonus, blood sugar normal, tissues bind calcium), the reaction is toward the alkaline side and the permeability of the capillaries is lessened; that is, the organism is in the state of sympathetic tonus, the blood sugar is normal and the tissues bind calcium.

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## THE EFFECT OF HIGH PROTEIN DIETS ON THE KIDNEY FUNCTION IN DOGS\*

LEO K. CAMPBELL, M.D., CHICAGO, ILL.

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THE object of the investigation discussed in this paper was to determine whether or not the renal and arterial disease described by L. H. Newburgh<sup>†</sup> in rabbits after feeding high protein diets could be produced in adult dogs. Dogs were chosen because their naturally selected diet more nearly approaches the human diet than that of any other laboratory animal.

### METHOD OF STUDY

Five disease-free female dogs were operated upon under ether anesthesia and a small block of tissue for microscopic examination was removed from the upper pole of the left kidney. After about four weeks the kidney function, as determined by qualitative analysis of the urine for albumin, blood, casts, and cells, the total nitrogen of the urine by the Kjeldahl method, the phenolsulphonphthalein dye test determined in a Klett biologic colorimeter, and the non-protein nitrogen of the blood, had returned to normal. The animals were then fed quantitative diets which contained 5 gm. of protein per kilogram body weight and afforded 50 calories per kilogram. This calorie intake was necessary because the dogs were permitted exercise except during the short intervals when they were in the metabolic cages. The diets were made up of ground meat, composed of seven-eighths round steak, one-sixteenth each of heart muscle and liver, and lard. To this diet was added 0.01 gm. of calcium as calcium carbonate and 0.30 of a minim of Meade's viosterol in oil 250-D per kilogram. Sufficient sodium chloride was added to season. This diet fulfills all the nutritional requirements and, of course, was readily taken by the dogs. The control was fed the stock laboratory diet. The feeding was continued for five months, at the end of which time the kidney function was again determined, the dogs reoperated upon, and tissue from the right kidney of each dog was removed for microscopic examination.

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\*From the Department of Medicine of Rush Medical College of the University of Chicago and the Presbyterian Hospital.

Received for publication, January 30, 1935.

†Newburgh, L. H., and Clarkson, Sarah: *Arch. Int. Med.* 32: 850, 1923.

## RESULTS

The figures in Table I indicate the kidney function at the beginning and at the end of the experiment.

Microscopic examination of the kidney tissue removed before the experiment and on the last day of the five-month feeding period revealed normal kidney parenchyma and vascular system.

TABLE I

DOG	DIET	WEIGHT (KG.)	AT BEGINNING OF EXPERIMENT				AFTER HAVING BEEN ON THE HIGH PROTEIN DIETS FOR FIVE MONTHS			
			QUALITATIVE ANALYSIS OF URINE FOR ALBUMIN, CELLS, BLOOD, CASTS	TOTAL NITROGEN IN URINE	NONPROTEIN NITROGEN OF BLOOD	PHENOLSULFONPHTHALEIN EXCRETION TEST	QUALITATIVE ANALYSIS OF URINE FOR ALBUMIN, CELLS, BLOOD, CASTS	TOTAL NITROGEN IN URINE	NONPROTEIN NITROGEN OF BLOOD	PHENOLSULFONPHTHALEIN EXCRETION TEST
I	CHO - P 32.4 F 21.9 Cal 325.0	6.5	Normal	5.2	0.028	65%	Normal	4.8	0.028	68%
II	CHO - P 48.2 F 33.3 Cal 493.0	9.6	Normal	6.8	0.036	61%	Normal	6.9	0.040	62%
III	CHO - P 56.2 F 34.0 Cal 530.0	10.8	Normal	9.0	0.035	62%	Normal	8.2	0.036	93%
IV	CHO - P 50.2 F 33.7 Cal 504.0	10.2	Normal	7.4	0.035	62%	Normal	7.8	0.028	90%
V (Control)	Stock diet	14.4	Normal	--	0.037	94%	Normal	---	0.029	84%

Two of the dogs went through pregnancy and gave birth to normal full-term pups without any evidence of kidney damage or toxemia. All of the animals were in excellent physical condition at the end of the experiment.

## CONCLUSION

Diets containing 5 gm. of protein per kilogram body weight and fulfilling all nutritional requirements when fed to adult dogs for five months produced no kidney damage that could be demonstrated by standard renal function tests or by microscopic examination. Attention may be drawn to the fact that five grams of protein per kilogram is four to five times higher than the average American adult consumption, and that five months in the life of a dog compares with two years in the human being.

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## THE EFFECT OF HIGH PROTEIN DIETS ON THE KIDNEY FUNCTION IN DOGS\*

LEO K. CAMPBELL, M.D., CHICAGO, ILL.

THE object of the investigation discussed in this paper was to determine whether or not the renal and arterial disease described by L. H. Newburgh† in rabbits after feeding high protein diets could be produced in adult dogs. Dogs were chosen because their naturally selected diet more nearly approaches the human diet than that of any other laboratory animal.

### METHOD OF STUDY

Five disease-free female dogs were operated upon under ether anesthesia and a small block of tissue for microscopie examination was removed from the upper pole of the left kidney. After about four weeks the kidney function, as determined by qualitative analysis of the urine for albumin, blood, casts, and cells, the total nitrogen of the urine by the Kjeldahl method, the phenolsulphonphthalein dye test determined in a Klett biologie colorimeter, and the non-protein nitrogen of the blood, had returned to normal. The animals were then fed quantitative diets which contained 5 gm. of protein per kilogram body weight and afforded 50 calories per kilogram. This caloric intake was necessary because the dogs were permitted exercise except during the short intervals when they were in the metabolic cages. The diets were made up of ground meat, composed of seven-eighths round steak, one-sixteenth each of heart muscle and liver, and lard. To this diet was added 0.01 gm. of calcinm as calcinm carbonate and 0.30 of a minim of Meade's viosterol in oil 250-D per kilogram. Sufficient sodium ehloride was added to season. This diet fulfills all the nutritional requirements and, of course, was readily taken by the dogs. The control was fed the stock laboratory diet. The feeding was continued for five months, at the end of which time the kidney function was again determined, the dogs reoperated upon, and tissue from the right kidney of each dog was removed for microscopie examination.

\*From the Department of Medicine of Rush Medical College of the University of Chicago and the Presbyterian Hospital.

Received for publication, January 30, 1935.

†Newburgh, L. H., and Clarkson, Sarah: *Arch. Int. Med.* 32: 850, 1923.

## RESULTS

The figures in Table I indicate the kidney function at the beginning and at the end of the experiment.

Microscopic examination of the kidney tissue removed before the experiment and on the last day of the five-month feeding period revealed normal kidney parenchyma and vascular system.

TABLE I

Dog	Diet	Weight (kg.)	AT BEGINNING OF EXPERIMENT				AFTER HAVING BEEN ON THE HIGH PROTEIN DIETS FOR FIVE MONTHS			
			QUALITATIVE ANALYSIS OF URINE FOR ALBUMIN, CELLS, BLOOD, CASTS	TOTAL NITROGEN IN URINE	NONPROTEIN NITROGEN OF BLOOD	PHENOLSULPHONPHTHALEIN EXCRETION TEST	QUALITATIVE ANALYSIS OF URINE FOR ALBUMIN, CELLS, BLOOD, CASTS	TOTAL NITROGEN IN URINE	NONPROTEIN NITROGEN OF BLOOD	PHENOLSULPHONPHTHALEIN EXCRETION TEST
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## CONCLUSION

Diets containing 5 gm. of protein per kilogram body weight and fulfilling all nutritional requirements when fed to adult dogs for five months produced no kidney damage that could be demonstrated by standard renal function tests or by microscopic examination. Attention may be drawn to the fact that five grams of protein per kilogram is four to five times higher than the average American adult consumption, and that five months in the life of a dog compares with two years in the human being.

## BLASTOMYCOSIS IN AN INFANT SIX MONTHS OLD\*

J. M. ROSENTHAL, M.D., ALLENTOWN, PA.

THE reason I am reporting this case is because of the age of the patient, and the rarity of the disease in this part of the country. This case was referred to our laboratories by a local physician who, after exhausting his medical and therapeutic knowledge, decided that laboratory studies should be undertaken in the interest of his patient.

According to Pussey's *The Principles and Practice of Dermatology*, blastomycosis is a specific infectious disease produced by a yeast fungus and characterized, as it occurs in the skin, by the formation of elevated, warty, suppurating lesions with abruptly sloping purplish red borders which are studded with pinpoint-sized deep-seated epidermal abscesses, from the pus in which pure cultures of the organism can usually be obtained.

The recognition of blastomycotic dermatitis as a pathologic entity dates from the meeting of the American Dermatological Association, in May, 1894, when Gilchrist demonstrated sections containing budding organisms which had been taken from a serofuloderma-like lesion.

In November, 1894, Busse reported a fatal case of pyemia with subcutaneous abscesses and cutaneous lesions in which the pathogenic agent was yeast.

July 7, 1894, Buschke (Greifswald Medical Society) referred to a case with skin lesions in which he found coecidial bodies. In Chicago the disease was studied by Ricketts, Hyde, Montgomery and Ormsby. The name of blastomycotic dermatitis was applied by Gilchrist. The name blastomycosis suggested by Hyde is perhaps better.

*Histology.*—The histologic features of this case were as follows: Proliferation of epithelial cells of the rete, formation of miliary abscesses within the epidermis and acute inflammatory changes in the corium. The cells of the rete were larger and swollen, and within the cells there were polymorphonuclear leucocytes, cellular detritus, some giant cells and a varying number of specific organisms.

The physician requested us to make a study of the scrapings from the epidermal abscess, from a microscopic and bacteriologic standpoint. We made several cultures and smears from the material in our effort to classify the mould and identify any organism which we might find. On microscopic study, we concluded that the causative organism was the *Cryptococcus gilchristi*, found in the purulent discharge as a round, doubly contoured budding yeastlike cell, 10 to 12 microns. In culture we saw the formation of a mycelium resembling that of an oidium.

\*From the Hamilton Laboratories.

Received for publication, February 14, 1935.

The media used was glucose agar, and after three days a white fluffy growth with aerial hyphae was observed. Stained with the thionin stain, mycelium was seen with sporelike bodies embedded in it.

The child had this condition for a period of three weeks, and after treatment with potassium iodide and x-ray applications, the child recuperated fully after six months' treatment.

This case was demonstrated at the Dermatological Clinic at Temple University, Philadelphia, and it was the first case which they had observed in a child so young. The diagnosis was confirmed at Temple University.

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814-816 GORDON STREET

## THE EFFECT OF FORMALDEHYDE ON THE GROWTH OF TUBERCLE BACILLI\*

RUSSELL D. HERROLD, CHICAGO, ILL.

THE sterilizing action of formaldehyde on nonspore-forming bacteria is well known, and this method is commonly used for sterilization of instruments or other equipment that may be damaged by other means.

Previously I had noted that the growth of certain pathogenic bacteria such as staphylococci and colon bacilli was inhibited or prevented after inoculation when the Petri dishes were placed in closed cans containing formaldehyde.

It seemed worth while to determine the relative resistance of tubercle bacilli to formaldehyde as compared to contaminating bacteria with the idea that such treatment might prove to be a satisfactory substitute for the acid or alkaline treatment of sputums or other suspected tuberculous material before inoculation of culture medium when such materials contained other bacteria.

Sputums positive for tubercle bacilli were selected for the experiment and the cultural observations were made on an egg yolk agar medium that I† have

\*From the John McCormick Institute for Infectious Diseases.  
Received for publication, January 21, 1925.

†Aided by a grant from the Fenger Memorial Fund.

previously described. I have found that when the egg yolk is added to the melted agar according to the technic described in the above reference, a very small percentage of contaminations result on the uninoculated plates or slants. This has been confirmed by Woolsey<sup>2</sup> and Feldman,<sup>3</sup> the latter of whom states that it was unusual for a tube to be lost on account of contamination when the egg yolks were used. I believe that the flaming of the shell through a Bunsen burner before opening destroys any sporogenous bacteria that may be dormant between the outer shell and inner membrane, whereas these organisms are not destroyed when the eggs are treated with phenol and alcohol as is commonly done in the preparation of Löwenstein's and other similar mediums. A pledget of cotton saturated with formaldehyde was placed in the bottom of the closed containers of two quart capacity which were used for exposure of the inoculated specimens.

Two parallel series were tested for the effect on the growth of the tubercle bacilli and other bacteria after exposure of the sputum inoculum to formaldehyde for periods of two and twenty-four hours, after which the inoculated plates were removed from the formaldehyde cans, incubated, and readings made at intervals for thirty days.

In the first series the sputum, positive for tubercle bacilli, was treated for one-half hour with 3 per cent sodium hydroxide, and three egg yolk agar plates then were inoculated heavily with an equal amount of centrifuged sediment. The control plate that was incubated without exposure to formaldehyde gave the first macroscopic appearance of tubercle bacillus colonies five days after inoculation, and by the ninth day there was a heavy growth. The plates subjected to formaldehyde for two hours at room temperature gave the first macroscopic appearance of growth on the ninth day, and reached its maximum growth on the eighteenth day, at which time there was about 50 per cent inhibition as compared to the control plate. The third plate was left in the formaldehyde container for twenty-four hours at room temperature, after which it was removed and placed in the incubator. There was no growth of tubercle bacilli or other bacteria during the thirty-day period of observation.

The second series consisted of inoculation of three plates with the same positive sputum which was not treated by sodium hydroxide. The control plate at the end of twenty-four hours was completely covered with a confluent growth of a mixture of gram-positive cocci and bacilli. The second plate which had been placed in the formaldehyde can for two hours before incubation gave a similar growth of flora as the control plate at the end of twenty-four hours, but there was approximately 50 per cent inhibition in the growth of the same mixture of gram-positive cocci and bacilli. The third plate which had been left in the formaldehyde can for twenty-four hours before incubation did not yield any tubercle bacilli or other bacteria during the thirty-day period of observation. This experiment was repeated with a second positive sputum with similar results. On the plates which had been subjected to the formaldehyde for two hours, there was a growth of tubercle bacilli equivalent to about 25 per cent that of the control of the first series treated by sodium hydroxide, and there was a comparative delay of about one week in the appearance of colonies.

## DISCUSSION

It is apparent from these observations that tubercle bacilli are relatively more susceptible to formaldehyde as compared to other bacteria than when treated with acid or alkalies in the strength commonly used for destruction of secondary bacteria in the cultivation of tubercle bacilli. Obviously the above observations indicate that formaldehyde is not a satisfactory substitute for the acid or alkali treatment of specimens before inoculation of culture mediums.

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## CONCOMITANT COMPLETE HEART-BLOCK AND BUNDLE-BRANCH BLOCK\*

### A THEORETICAL DISCUSSION

EMMET FIELD HORINE, M.D., AND MORRIS M. WEISS, M.D.,  
LOUISVILLE, KY.

ADMITTEDLY there is every reason to believe that complete auriculoventricular dissociation and bundle-branch block may coexist. However, a concomitant diagnosis of these two lesions cannot be made in every instance that the electrocardiogram seems to indicate the association. This theoretical discussion appears advisable in view of the frequency with which such a combined diagnosis is made unreservedly.<sup>1-5</sup>

Even though experimental bundle-branch block produces such a characteristic electrocardiogram that there can be no reasonable doubt that a similar human electrocardiogram must indicate bundle-branch involvement, there are a few conditions which must be excluded. Paroxysmal ventricular tachycardia produces complexes similar in form to bundle-branch block. Likewise paroxysmal auricular tachycardia with the impulse pursuing an aberrant course in the ventricle might resemble auricular tachycardia with a bundle block. Finally the idioventricular rhythm in complete A-V dissociation *due* to a focus of impulse formation *below the main stem* of the bundle of His would resemble that of branch block. If, in complete heart-block, an idioventricular impulse originates

\*From the Division of Medicine, School of Medicine, University of Louisville.  
Received for publication, February 16, 1935.



in the main stem but is blocked in the bundle or if the focus is *below the* main stem, the end-result of the spread of the contraction over the ventricles would be the same and produce similar electrocardiographic findings.

The combined diagnosis of complete heart-block and bundle-branch block is not warranted without excluding a rhythm originating below the division of the main stem. In complete dissociation of the auricles from the ventricles the idioventricular rhythm usually originates in the main bundle below the site of the injury. Of this the electrocardiogram furnishes proof. The normal ventricular complex is preserved, being the same both before and after the injury. This fact indicates that the impulse arises somewhere between the lesion and the point at which the bundle divides into its right and left branches.<sup>9</sup> Hence, in a slow idioventricular rhythm with bundle-branch type of QRST complexes it is ordinarily assumed that the idioventricular focus is in the main stem and that there is a coincident lesion of one of the branches which accounts for the abnormal complexes. However, aberrant ventricular complexes in complete block must be critically interpreted because portions of the ventricle, other than the His bundle, are capable of initiating an idioventricular rhythm. Lewis<sup>9</sup> states: "In the ventricles, the bundle is certainly endowed with rhythmic power; its continuation in branches of similar elemental constitution in dog and man suggests that these may possess similar properties. It is said that the main divisions readily become rhythmic on heating and we know that when both divisions of the bundle are cut the ventricle continues to respond to impulses generated within itself. The cells of the arborization also possess rhythmicity. . . ." Idioventricular pacemakers, other than those in the main stem, would produce complexes resembling those found in bundle-branch block or even with more diverse and bizarre features. It is apparent, therefore, that the concomitant diagnosis of complete A-V dissociation and bundle-branch block cannot be made unreservedly.

Failure to make a critical differential diagnosis may explain the frequency with which such a concomitant diagnosis has been made.<sup>1-8</sup> Ellis and Weiss<sup>10</sup> included four cases of bundle-branch block among forty-three cases of complete A-V block. In one case the original electrocardiogram showed complete heart-block with left bundle-branch block. A few days later another electrocardiogram resembled the preceding except that right bundle-branch block now existed. They state: "A possible explanation for the development of first left and then right bundle-branch block in successive electrocardiograms is that the origin of the impulse which excited the ventricular contraction was first in one branch of the bundle of His and subsequently shifted to the other branch." In a recent study of 155 cases of bundle-branch block, King<sup>12</sup> reported an associated complete A-V block in thirty-six instances. He noted in such cases a tendency for the bundle block to shift from side to side, and in one case it was thought that the origin of the impulse was not in the main stem below the site of the A-V block but probably first in one ventricle and subsequently in the other. In the light of our discussion above we seriously question whether one would be justified in reporting such combined diagnoses without pathologic confirmation or qualifying reservations.

A definite diagnosis of combined lesions might be made from comparative records. Thus, if a patient with bundle-branch were observed to develop complete A-V dissociation and the bundle-branch complexes persisted, it would be reasonable to assume that two entities were present. Also, if a patient with complete heart-block exhibited a change from supraventricular to bundle-branch complexes, a combined diagnosis would seem justified.

# CONCLUSION

We believe that it is theoretically impossible, from a single record, to make a concomitant diagnosis of bundle-branch block and complete heart-block.

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## HIGH BLOOD UREA NITROGEN NOT DUE TO CHRONIC NEPHRITIS

MICHAEL G. WOHL, M.D., AND RAYMOND W. BRUST, M.D.,  
PHILADELPHIA, PA.

THE impression gained from a perusal of the literature, as well as from modern textbooks of medicine, is that an increase in the nitrogenous waste products in the blood is indicative of chronic glomerular nephritis; this notwithstanding the appearance in recent literature of reports of high blood urea nitrogen not associated with chronic kidney disease.

The importance of recognizing these states clinically and early lies in the fact that many of them are mistaken for coma of renal origin, especially since traces of albumin and some casts are not infrequent urinary findings and furthermore because immediate proper treatment of the nonrenal cases has often proved life-saving.

A glance at Table I will indicate the common findings in the two conditions which serve to differentiate them.

Up to the present time a variety of clinical conditions with which high blood urea or nonprotein nitrogen values are found have been reported:

1. Vomiting from many causes:
  - a. Cancer of the stomach<sup>1</sup>
  - b. Pylorospasm<sup>2</sup>
  - c. Hyperemesis gravidarum<sup>3</sup>
  - d. Gastric tetany<sup>4</sup>
  - e. Alkalosis in alkaline treatment of peptic ulcer<sup>5</sup>
  - f. Experimental pyloric occlusion<sup>6</sup>
  - g. Acute intestinal obstruction<sup>10, 11, 12, 13</sup>
  - h. Acute general peritonitis<sup>14</sup>
2. Repeated gastric lavage<sup>6</sup>
3. Diarrhea<sup>7, 8</sup>
4. Acute cerebral accidents<sup>9</sup>
5. Reflex anuria<sup>9, 15</sup>
6. Diabetes mellitus<sup>16</sup>
7. Hyperparathyroidism<sup>17</sup>
8. Extensive burns<sup>18</sup>
9. Pancreatic necrosis<sup>19</sup>
10. Hypersensitiveness<sup>20</sup>
11. Infectious diseases<sup>21</sup>

In addition to the above we have had under observation a number of cases with conditions illustrating the phenomenon of high blood urea nitrogen, some not hitherto reported.

CASE 1.—Vomiting associated with obstipation. Mr. M. B., aged seventy-three years, admitted March 7, 1934, to the Philadelphia General Hospital, service of Dr. Wm. E. Robertson, with a chief complaint of vomiting and obstipation.

*History.*—Prior to the onset of the present illness the patient had been troubled with obstinate constipation. On March 1, 1934, he began vomiting everything he ate, and this condition persisted for a week. The vomitus consisted of a yellow muddy fluid combined with whatever he had eaten. The vomiting occurred at frequent intervals as well as immediately after the ingestion of food. He was able to retain only small amounts of tea and toast. The patient had no bowel movement for several days in spite of the use of citrate of magnesia and castor oil. He was not completely bedfast, but he steadily was growing weaker.

TABLE I

	CHRONIC GLOMERULO- NEPHRITIS	NONRENAL AZOTEMIA
History of preceding nephritis	May be present	Absent
Blood pressure	Increased	Usually normal or low
Eyegrounds	Usually show exudate hemorrhage or vascular changes	Negative
Respiration	May be Cheyne-Stokes	Usually shallow and rapid but regular
Urine	Acid, low fixed specific gravity, albumin and casts, erythrocytes usually present. Chlorides not significantly altered	Alkaline in hypochloremic type. Sp. gr. variable, usually high. Albumin and casts may be present but disappear with improvement in clinical condition. Erythrocytes not abnormal. Chlorides diminished or absent
Kidney function tests	Always show marked impairment of renal function	May show impairment at height of disorder—improvement occurs with clinical improvement and decrease of blood urea nitrogen
Blood chlorides	Not altered significantly (normal or slightly reduced)	Diminished in hypochloremic coma. Variable in other cases
CO <sub>2</sub> combining power of blood plasma	May be markedly diminished	Increased in hypochloremic cases. Normal or decreased in other types
Blood count and hemoglobin	Reduced	Normal or elevated—anhedremia. One exception in our toxic case—where there was a severe anemia
pH of blood	Usually low	Usually high
Therapy	Course of disease not influenced by administration of salt and fluids	Salt and fluid administration curative in early stages

The man had been in good health previous to his present illness, with no special complaint except constipation for which various laxatives were used. In the two years before the present illness there was no loss of weight, but within a week on becoming sick, the loss was considerable.

*Examination.*—Here was an elderly man in a deplorable state—there was pronounced emaciation; the skin was dry; the tongue was dry and moderately coated; the eyes were sunken; the man was mentally confused and somnolent.

The blood pressure was 120 systolic and 70 diastolic. The brachial vessels were tortuous and the pulsations visible. Some thickening of the radial vessels was noted.

The abdomen was greatly distended and borborygmus was audible. There was no reverse peristalsis. The size of the liver was within normal limits. The spleen was not palpable. The prostate was large, smooth and slightly tender. No rectal masses were palpable.

The temperature was 98° F. The pulse rate was 80 and the respirations were 25. The tendon reflexes were normal.

The laboratory findings were: blood sugar 132 mg. per cent, blood urea 120 mg. per cent, creatinine 6.2 mg. per cent. The urine was acid in reaction, had a specific gravity of 1.020 and showed a trace of albumin but no sugar. On microscopic examination two-plus leucocytes were reported. The amount of urine voided was diminished.

*Treatment.*—It was suspected that either an intestinal obstruction or an acute uremia was the cause of the trouble, and therefore an attempt was made to give the man a barium enema but without success. He was then given 3,500 c.c. of 3 per cent glucose in normal salt solution subcutaneously and within twenty-four hours the vomiting stopped. This procedure was repeated the following day. The patient became able to retain fluids by mouth and his mentality began to clear.

The blood chemistry seven days after his admission showed a blood urea nitrogen of 100 mg. per cent, a blood sugar of 104 mg. per cent, and a creatinine of 2.4 mg. per cent. Large amounts of glucose and saline were given by rectum in addition to subcutaneous saline administration. The diuresis increased considerably. On the fourteenth day after admission the blood chemistry showed: urea nitrogen 50 mg. per cent, and creatinine 2.0 mg. per cent. By March 23, improvement continued and the patient was able to sit up in a chair and he was mentally quite clear. By March 25 he was taking food freely and doing well. The kidney function tests and urinalysis were about normal, and the patient was discharged.

**CASE 2.**—Vomiting following self-treatment with a duodenal tube. Mrs. K. C., aged forty-four years, was examined by one of us (M. G. W.) at the Temple University Hospital, sixteen months prior to her present illness. A diagnosis of noncalculous cholecystitis and general visceroptosis was made. The patient had several so-called nonsurgical gallbladder drainages. She then, without the physician's permission or knowledge, procured a Rehfuß tube which she used whenever nauseated or had, as she put it, a "bilious attack."

On May 5, 1934, the patient vomited a large quantity of dark green material. This vomiting continued for the following five days and she was unable to retain anything on her stomach. She continued to use the Rehfuß tube for the next four days. On the ninth day she suffered a violent headache; she became extremely weak and somnolent. When the woman was seen she looked emaciated; her skin was dry, her eyes were sunken, but the tongue was moist, though coated. The breathing was rapid but shallow and faint breath sounds were heard over the lungs. The heart was rapid and the sounds weak. The blood pressure was 90 systolic and 70 diastolic. The abdomen was greatly distended. The tendon reflexes were retained.

The urine showed a trace of albumin, many cylindroids, and a number of leucocytes. There were practically no chlorides in the urine. The blood chemistry showed: urea nitrogen 75 mg. per cent and chlorides 420 mg. per cent.

*Treatment.*—Bismuth carbonate gr. v and cocaine hydrochloride gr.  $\frac{1}{4}$  were given every two hours until the gastric irritability was allayed. Rectal administration of saline and 3 per cent glucose was adopted, and in addition the patient was given highly salted bouillon and was instructed to chew salty fish. After twelve hours of this salt diet, fluids and some food could be retained. The woman gradually became stronger and she continued to improve.

Two weeks later the urine showed the presence of chlorides; the blood urea had declined to 22 mg. per cent and the blood pressure had risen to 115 systolic and 80 diastolic, and the patient was able to be up and about.

**CASE 3.**—Diarrhea. A negro laboring man, aged forty-eight years, was admitted to the service of Dr. David Riesman at the Philadelphia General Hospital on Sept. 9, 1934, with a chief complaint of great loss of weight, severe weakness and diarrhea. He was having six to ten bowel movements a day. His trouble began with a chill while at work as a stevedore.

Examination showed a greatly dehydrated, emaciated, dull and drowsy negro in a critical condition. There was no marked dyspnea, no cyanosis or edema. The temperature was 95° to 97° F. The blood pressure was 97 systolic and 82 diastolic. The blood count showed the erythrocytes to be 4,700,000, the hemoglobin 70 per cent, and the leucocytes 7,300. The urine was acid in reaction. There was no albumin and no sugar; but some hyaline casts and leucocytes were reported. The blood chemistry showed the urea nitrogen to be 72 mg. per cent and the creatinine 2.6 mg. per cent.

*Treatment.*—Hypodermoclysis of normal salt to which was added 50 c.c. of a 50 per cent glucose solution per 1,000 c.c. was given frequently. Bismuth and paregoric and atropine were administered by mouth.

The man began to react at once and steadily improved. No specific lesion or organisms were discoverable in the bowel. On September 28, ten days after admission, his blood urea nitrogen was only 26 mg. per cent and before his discharge it had fallen to 18 mg. per cent. Unfortunately the chlorides were not determined either in the blood or in the urine.

CASE 4.—Vomiting and diarrhea from intestinal obstruction. A. M., a colored man aged forty-four years, a laborer, admitted to the Philadelphia General Hospital on Nov. 29, 1934, with the complaints of vomiting bloody fluid and pains in the abdomen which began the day before admission three hours after last drinking considerable cheap whisky. At 7 P.M. on November 28 he began to have cramps, and he vomited a "gallon" of brownish black stuff. By midnight he had twelve stools in quick succession without blood. The pain continued.

His past history revealed that he had been operated upon for appendicitis and that "at the same time he had an ulcer cut out through a higher incision."

Examination showed a middle-aged negro man who was restless, slightly cyanotic in his lips, and malnourished. He was oriented, intelligent and cooperative. The skin was warm and moist. The face had an anxious and pinched expression. The abdomen had increased resistance to palpation and tenderness in the upper half. There was no distention and no fluid. The blood pressure was 110 systolic and 80 diastolic. The urinalysis showed an acid reaction, a specific gravity of 1.020. There were two-plus albumin and two-plus leucocytes but no sugar. Blood erythrocytes were 3,530,000; leucocytes 9,400.

By November 30 the patient was very dehydrated. Intestinal obstruction was diagnosed and conservative treatment by the Wangenstein suction siphonage method was instituted. On December 1 the erythrocytes numbered only 2,440,000 with the hemoglobin reading 50 per cent. The blood chemistry was: sugar 109 mg. per cent, urea nitrogen 115 mg. per cent, creatinine 4.5 mg. per cent, and chlorides 374 mg. per cent.

On December 1 he was given salt solution hypodermically and glucose intravenously. He became almost pulseless. The same day he was given 500 c.c. of citrated blood. The next day he improved; the duodenal tube was withdrawn on December 3, but he started to vomit again in spite of continued parenteral salt and sugar solutions.

On December 4 he was operated upon and twelve inches of a jejunum which had been anastomosed to the stomach were found intussuscepted into the latter which was enormously distended. Parenteral salt and sugar solutions were continued. By December 5 his blood urea nitrogen was 60 mg. per cent and the creatinine 2 mg. per cent. The chlorides were now 590 mg. per cent. On December 15, with continued clinical improvement, the urea nitrogen was 12 mg. per cent and the chlorides 545 mg. per cent.

Postoperative hyperazotemia and hypochloremia have already been commented on in the literature<sup>21, 22</sup> and hypertonic (4 per cent) solutions of salt have been recommended intravenously to combat accidents due to these states. That such conditions do sometimes prevail after operation, and the importance of properly diagnosing the pathologic physiology, are the reasons for including the following case.

CASE 5.—Postoperative dehydration. Mr. A. B., a white man aged seventy-three years, a known mild diabetic (not requiring insulin) of at least two years' duration, was admitted

to the Jefferson Hospital on Sept. 24, 1934, on account of acute complete urinary retention from benign hypertrophy of the prostate. His general condition was good for a man of his age.

The laboratory findings on admission were: urine, alkaline in reaction; specific gravity, 1.010; albumin, negative; sugar and acetone, negative. Red blood cells were found as well as an occasional leucocyte. There were no casts. Blood: the count showed erythrocytes, 4,750,000; leucocytes, 9,200; color index, 1.02; hemoglobin, 96 per cent. The Kahn test was negative. Chemistry: urea nitrogen 21 mg. per cent, and sugar 193 mg. per cent. The urine examination subsequently showed a variation in specific gravity from 1.010 to 1.020. On only one occasion, Sept. 16, 1934, was there any albumin, and then a cloud was reported present.

A two-stage prostatectomy was done, the second stage under spinal anesthesia on Oct. 6, 1934. After the first stage the blood sugar was kept between 116 and 129 mg. per cent. On Oct. 3, 1934, the blood nonprotein nitrogen was 29.85 mg. per cent, and the urea nitrogen was 19.85 mg. per cent.

Following both stages glucose 5 per cent in 1,000 c.c. of normal salt solution was given intravenously with one unit of insulin for every gram of glucose. During the second stage there was a severe reaction from the spinal anesthesia but this responded to epinephrine hydrochloride, 1 c.c. of the 1:1,000 solution and ephedrine sulphate  $\frac{3}{4}$  gr. hypodermically.

In the two days following, the patient did not take fluids as he should have and on October 8 his blood nonprotein nitrogen rose to 50 mg. per cent with the creatinine 1.72 mg. per cent, and the patient became drowsy and his skin was dry. Fluids were pushed and by October 11 the nonprotein nitrogen had come down to 33 mg. per cent.

This and another almost identical case (nondiabetic) were at first regarded as impending renal failure following prostatectomy until the true background of the sudden elevation of nonprotein nitrogen was evaluated and counteracted by forcing fluids. Without the immediate postoperative intravenous infusion, no doubt the condition would in both of these cases have been far more alarming, especially on account of the nature of the condition.

Congestive heart failure with passive congestion of the kidneys has been mentioned in the literature as a possible, though by no means likely, cause of great elevation of blood urea nitrogen. Tileston and Comfort<sup>10</sup> reported a study of a series in which one case showed an elevation of 71 mg. per cent. Cases, however, have been known<sup>23</sup> where, due to congestive failure, the clinical picture so resembled uremia of renal origin that the patient was about to be given a hot pack until the cardiac origin of the renal symptoms was stressed. The renal picture reverted to normal on reestablishment of circulatory competence. A milder case came under the observation of one of us (R. B.) recently.

CASE 6.—M. S., a negro man aged forty-four years, with congestive heart failure from hypertensive and/or syphilitic heart disease. He was first admitted to the Philadelphia General Hospital on March 18, 1934, with moderate congestive heart failure and a blood pressure of 160 systolic and 120 diastolic.

The urine showed an acid reaction, a specific gravity of 1.016 to 1.030, a trace of albumin and, on microscopic examination, there were occasional erythrocytes, granular casts and leucocytes in the first specimen only.

The electrocardiogram showed definite evidence of myocardial disease.

The man improved steadily and was discharged only to be readmitted on Nov. 2, 1934, in a worse state than the first time. He had anasarca, a large liver, bilateral hydrothorax and Cheyne-Stokes breathing.

The urine was acid, the specific gravity was 1.015 to 1.030 with, at first, a heavy trace of albumin and some casts. When his condition improved the albumin and casts were no longer present.

On this admission the blood urea nitrogen was 42.0 mg. per cent and the creatinine 2.0 mg. per cent and this urea nitrogen came down to 29.0 mg. per cent after his congestive failure was relieved and he improved generally.

CASE 7.—More severe acute (toxic?) kidney damage apparently not associated with any previous renal lesion or even any glomerular damage is illustrated by the following unusual case:

A colored man, J. D., aged twenty-four years, was admitted to the Philadelphia General Hospital on June 24, 1934, for headache, vomiting, and inability to move his bowels well. There was a gradual onset preceded by a chest "cold" and the drinking of a half pint of "corn whisky." He had nocturia, frequency and dysuria since the onset. He had an acute gonorrheal urethritis one month before.

Examination showed a well-nourished and well-developed young negro man with warm sweating skin and a fever of  $101^{\circ}$  to  $103^{\circ}$  F. His blood pressure was 110 systolic and 78 diastolic. He was passing cloudy urine. The man was at first thought to have an intestinal obstruction and was sent to the surgical ward where the report of a blood urea nitrogen of 105.0 mg. per cent suggested uremia, and he was transferred to the medical ward. There were no oliguria, hematuria, or pyuria but there were urinary frequency, urgency, and slight burning on micturition. There were no casts in the urine until July 3, 1934, and the specific gravity ranged from 1.010 to 1.016. At first there was a heavy cloud of albumin but this disappeared by July 4, 1934. On July 3 it was noted that "in spite of daily intravenous glucose his blood urea nitrogen had increased rapidly and progressively" (to 230 mg. per cent). There was urea frost on his face; he was toxic and apathetic.

On July 8 a twenty-four-hour specimen of urine showed a trace of arsenic. By July 10 he had a blood pressure of 140 systolic and 90 diastolic. The phenolsulphonaphthalein test showed no elimination of the dye and the Mosenthal test showed a low fixed specific gravity and a night to day ratio of 4/1. The eyegrounds showed nothing abnormal. The blood count showed a severe anemia, the erythrocytes being 1,680,000 and the leucocytes 7,200 with a normal differential count.

On July 22 the man was given an indirect blood transfusion of 200 c.c. By July 28 he felt better and his blood urea nitrogen was only 32 mg. per cent. By August 12 the blood pressure was 130 systolic and 80 diastolic. The phenolsulphonaphthalein test showed an output of the dye of 14 per cent in the first hour and 16 per cent in the second hour. He continued to improve and was discharged in good condition with a blood urea nitrogen of only 22.0 mg. per cent. The diagnosis was acute toxic nephrosis.

Table II shows that the blood chlorides in this case were not significantly reduced but that at the height of his blood urea nitrogen he had a marked acidosis.

TABLE II  
BLOOD CHEMISTRY

DATE	SUGAR	UREA N	CREATIN- INE	BLOOD CHLORIDES IN TERMS OF NA CL	CO <sub>2</sub>	SERUM PROTEIN
6/26/34	109	105				
6/28/34	107	150	11.2	586		
7/ 3/34	125	230				
7/ 6/34	114	240	16.0		19 vol. %	Total 6.50 {albumin 3.18 globulin 3.32
7/10/34						
7/21/34	82	32				
7/28/34	96	23				
8/13/34	84	22				

This case was doubtless one of severe renal function impairment but on the other hand, not the type commonly associated with azotemia and more amenable to therapy.

CASE 8.—Epilepsy. Miss L. R., aged twenty-three years, admitted to the Temple University Hospital, Nov. 11, 1933, service of Dr. Temple Fay. The chief complaint was convulsive seizures. The history stated that the girl had suffered her first seizure at the age



of sixteen and since that time there had been one or two a month. These seizures seemed definitely related to the menstrual cycle occurring especially at the onset of the menstruation. They lasted from a few minutes to an hour and were followed by headache.

The case had been diagnosed as one of essential epilepsy, and the patient had been on a fluid limitation and salt-restricted diet for a period of two years. The night before admission to the hospital there were several convulsive seizures, and the following day eight of them. Amytal was given and oxygen inhalations were administered. Two days after admission the patient was stuporous, responding to pinprick only; she did not reply to questions, the pupils were contracted and responded only slightly to light. Physical examination was otherwise irrelevant. The eyegrounds showed no abnormalities.

On November 16 the blood chemistry showed a remarkable finding: the nonprotein nitrogen was 180 mg. per cent and the urea nitrogen was 95 mg. per cent, the creatinine was 7.3 mg. per cent, and the blood sugar was 135 mg. per cent.

The patient was given 3,000 c.c. of normal salt solution intravenously; she reacted well to the saline and was able to answer questions intelligently and to recognize her relatives.

On November 17 the patient's blood count showed a hemoglobin of 14.5 gm. per 100 c.c.

The red blood cells were 4,450,000 and the white blood cells were 17,900. The differential showed young forms 38 per cent, neutrophils 35 per cent, lymphocytes 16 per cent, and monocytes 11 per cent. Urinalysis showed a moderate trace of albumin, but no sugar. Microscopically there were found an occasional hyaline cast and a few white blood cells. The sedimentation time of the red blood cells was within normal range. The spinal fluid had a pressure of 8 mm. Hg and the chlorides were 775 mg. per cent.

The patient continued to be in a semistuporous state with a blood pressure of 95 systolic and 55 diastolic. The pulse rate was 120 per minute and the respiratory rate was 30 per minute. The temperature remained elevated, the highest point reaching 104° F. in the afternoons of the thirteenth and fourteenth of November.

On November 18 the blood urea was 100 mg. per cent, creatinine was 1.7 mg. per cent, and the chlorides were 520 mg. per cent. On that date she again lapsed into coma and was given 500 c.c. of 10 per cent glucose in saline solution intravenously. In addition 1,000 c.c. of saline were given by hypodermoclysis. This treatment was repeated on the following day, and the blood chemistry was then as follows: urea nitrogen 83 mg. per cent and creatinine 1.6 mg. per cent.

On November 21 the patient reacted from coma, answered questions intelligently and was quite cooperative. She complained of being weak. On this date the blood chemistry was: nonprotein nitrogen 56 mg. per cent and urea nitrogen 23 mg. per cent. The temperature had dropped to 100°, the pulse was 70 per minute, and the respirations were 20 per minute.

On November 23 the patient became quite alert and active. The temperature returned to normal and her general condition was good. The blood urea nitrogen on this date was 17 mg. per cent.

On December 1 the blood urea nitrogen was 9.0 mg. per cent and the patient became entirely free from symptoms (Table III).

TABLE III  
SHOWING CHEMICAL CONSTITUENTS OF THE BLOOD IN CASE 8

DATE	NONPROTEIN NITROGEN	CHLORIDES	UREA NITROGEN
Nov. 17, 1933	180		95
Nov. 18, 1933		520	100
Nov. 20, 1933			83
Nov. 21, 1933	56		23
Nov. 23, 1933			17
Dec. 1, 1933			9

The electrocardiogram taken during the height of the coma and subsequent to it is worthy of note. During the coma the electrocardiogram showed a slight depression of the S-T interval in Lead II, and this changed on the following day to an inverted T-wave with a depression of the S-T interval. With recovery of the patient the T-wave tended to become flat and later upright.

On December 4 the patient had two convulsive seizures each lasting two minutes and associated with involuntary voiding. She again suffered seizures on the tenth and fifteenth of December. She was discharged from the hospital on Dec. 19, 1933.

#### DISCUSSION

An analysis of the conditions with which high blood urea nitrogen is associated will reveal the similarity of certain types.

1. Loss of body fluids and depletion of blood electrolytes: (a) by vomiting, by bowel, or by skin. (b) Deficient intake: starvation, deprivation of salt and water.

2. Disturbances in the kidneys themselves: (a) acute intoxication and (b) passive congestion.

3. Neurogenic? (a) reflex anuria and (b) central cerebral?

4. Miscellaneous and little understood disturbances of metabolism.

A review of the cases reported will indicate that by far the commonest clinical cases fall into the first group, all but two of our cases.

Soon after Folin's method of blood analysis came into general use, Tileston and Comfort in 1914<sup>10</sup> reported a study of a miscellaneous series of cases in whom they had made estimations of the blood urea nitrogen and found elevations. They had three cases of acute intestinal obstruction. Cooke, Rodenbaugh and Whipple, in 1916 reported<sup>24</sup> experimental confirmation of high blood urea nitrogen values and more particularly high values for noncoagulable nitrogen in dogs with ligated loops of bowel. In 1919 Porges<sup>25</sup> reported clinical cases of associated high blood urea nitrogen and low blood chlorides. Later (1923) Brown and his associates<sup>26</sup> presented cases of high intestinal obstruction with the clinical picture of shock and coma. These observations were duplicated and further elaborated by Haden and Orr<sup>11</sup> in 1923 and by Whipple, Cooke and Staers in 1927.<sup>27</sup> High  $\text{CO}_2$  combining power of the blood was observed by these workers and their animals quickly improved when sodium chloride was administered.

Previously, in 1908, Grunwald<sup>28</sup> had reported that dogs on diuretin and a salt-free diet developed a marked depletion of body chlorides and that as a result the animals went into coma from which they died unless salt was given them.

The symptoms the patient shows depend partly upon the conditions underlying the loss of chlorides and partly upon the state of dehydration. Loss of weight, asthenia, decline of blood pressure, rise in temperature, dryness of the skin, and shallow respirations portray the clinical picture of hypochloremic coma. The patient may also become disoriented, somnolent, and should the chlorides greatly diminish, perhaps comatose. The blood is anhydremic with a high hemoglobin and an increase in red blood cells per unit volume. The non-protein and urea nitrogen are high. The blood chlorides are perhaps diminished but may be normal, especially in the early stages. The carbon dioxide-combining power of the blood plasma is increased, the urine may show albumin and casts, but a diminution or total absence of chlorides is the most striking finding. Neither the level of the blood urea nitrogen nor of the blood chlorides always determines the onset of coma; this will probably depend upon the constitutional predisposition of the patient, especially with reference to the nervous system.

The important thing is to recognize the condition because the administration of salt solution is so valuable in restoring the patient quickly to a normal state. For its recognition no elaborate procedures are necessary. After all, as Peters<sup>29</sup> states, the clinical estimate of dehydration, dry inelastic skin and mucous membranes, loss of panniculus, inelastic eyeballs, scanty high-colored urine of high specific gravity, etc., are more accurate than laboratory procedures, and the history is usually clear. Furthermore, for the general practitioner the examination of the urine for chlorides and of the blood for hemoglobin when added to the clinical picture will usually suffice to estimate fairly accurately such a case.

Concerning Types II, III, and IV, less definite information is at hand and general principles will have to guide the diagnosis and conduct of such a case.

The association, infrequent as it may be, of severe functional kidney impairment in passive congestion should be borne in mind as well as the recognition of milder phases in most instances of circulatory failure with some evidence of renal damage. These usually clear up more or less completely with restoration of cardiac competency.

Just why the urea nitrogen rises is not clear any more than is the wonderful normal blood regulatory mechanism. "The nonprotein nitrogen content of the blood may be considered as the resultant of three factors: the rate of destruction of protein in the body, the concentrating powers of the kidneys and the amount of water excreted. If the destruction of nitrogen is great enough, as it may be in such profound intoxications as lobar pneumonia, the blood nonprotein nitrogen may rise even when the kidneys are sound, especially if water excretion is small because the patient has received too little fluid."<sup>20</sup>

The story would not be quite complete without mentioning the many points of similarity between hypochloremic coma and the crises of Addison's disease. In both the prominent signs are: weakness, prostration, fall in blood pressure, high blood urea values and low blood chlorides. Sodium chloride alleviates to a striking degree these symptoms and findings in both Addison's disease and hypochloremic states. Out of the many functions, proved and unproved, assigned to the adrenal cortex one fact stands out, namely, that the hormone of the adrenal cortex exerts an influence on sodium metabolism and thus has a regulatory effect on salt and water metabolism.

#### CONCLUSIONS

1. Attention has been called to clinical states of high blood urea nitrogen values not due to chronic glomerular nephritis.

2. The importance of early recognition of this condition and the prompt improvement with proper therapeutic measures in certain types are illustrated by a number of cases.

3. The mechanism of nitrogen retention in these cases is not well understood, but it would appear to be intimately related in a majority of instances to alterations of water metabolism.

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## THE CARDIOVASCULAR SYSTEM IN PROTECTED AND UNPROTECTED ANIMALS WITH ACUTE DIFFUSE PERITONITIS\*

BERNHARD STEINBERG, M.D., AND J. LESTER KOBACKER, M.D., TOLEDO, OHIO

IN A PREVIOUS publication,<sup>1</sup> one of us produced experimental evidence that death in acute diffuse peritonitis is due to soluble toxic substances elaborated by bacteria present in the peritoneal cavity which are responsible for the peritonitis. This contention was based on experiments in which the peritoneal inflammation was induced by intraperitoneal injection of diphtheria bacilli suspended in gum tragacanth. Those animals which were given subcutaneous or intravenous diphtheria antitoxin invariably survived in spite of a marked peritonitis. Animals without antitoxin invariably succumbed.

Holzbach<sup>2</sup> in his review on circulatory failure in infectious conditions states that functional heart failure appears early in peritonitis, but this opinion is apparently based on clinical observation, since no experimental work on this condition was reviewed.

Two types of peritonitis were induced in dogs in our experiments: Fecal peritonitis by the intraperitoneal introduction of 5 gm. of small and large bowel feces suspended in 25 c.c. of saline; and, colon bacillus peritonitis by intraperitoneal injection of 50 c.c. of a 2 per cent gum tragacanth containing 200 million *B. coli* per c.c. Electrocardiographic and carotid pressure tracings on the kymograph before the onset and during the course of the peritonitis were the two criteria employed to determine the effect upon the heart. Two series of animals were employed: one series of twelve dogs in which no protection of any type was administered to the animals; in the other series of six animals all the dogs were protected by the administration intraperitoneally twenty-four to forty-eight hours before the onset of peritonitis of a suspension of killed colon bacilli in 1 per cent gum tragacanth (for the sake of brevity the material will be referred to as "coli-bactragen"). Previous publications<sup>3</sup> describe in detail the technique and the basis of this method of protection with coli-bactragen. It was desired to determine whether such a protection will evince any change which may otherwise be present in the unprotected animals on the electrocardiographic and kymographic tracings. All the animals were under pentobarbital sodium anesthesia. Along with each animal with peritonitis, a normal control dog was run simultaneously.

### THE EFFECT OF PERITONEAL INFECTION ON BLOOD PRESSURE IN PROTECTED AND UNPROTECTED ANIMALS

The effect on the blood pressure in the animals with fecal and *B. coli* peritonitis was alike. Within fifteen to twenty minutes after onset of the in-

\*From the Department for Medical Research, Toledo Hospital.  
Received for publication, February 27, 1935.

fection, the blood pressure began to drop gradually. In one hour there was a drop of 2 mm. of mercury. The fall was of the systolic pressure, the diastolic remained normal. The pulse pressure became progressively smaller. In six hours, the pressure dropped 5 mm. of Hg and at the same time an arrhythmia appeared. In the colon bacillus peritonitis, the fall was still more rapid although it seldom exceeded 4 mm. of mercury.

In the animals protected with coli-bactragen twenty-four to forty-eight hours prior to the onset of peritonitis, the systolic pressure dropped 1 mm. of mercury within ten to fifteen minutes and at the end of ten hours there was a total drop of 2 mm. of mercury. This drop in the protected animals was invariably a third

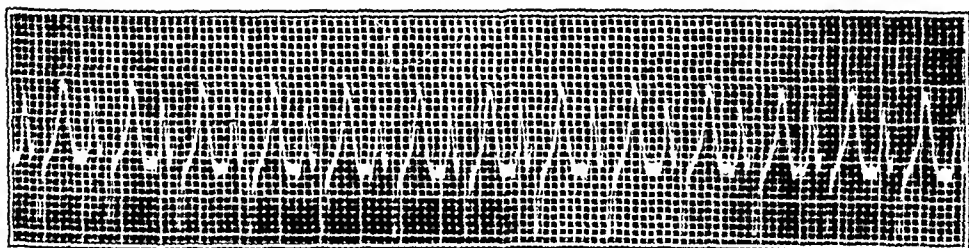


Fig. 1. Electrocardiographic tracing, one-half hour after induction of peritonitis in an unprotected dog. The pulse rate is 200. (An increase from a normal of 90.) There is some depression of the ST-interval as well as in all the subsequent figures Lead II is shown.

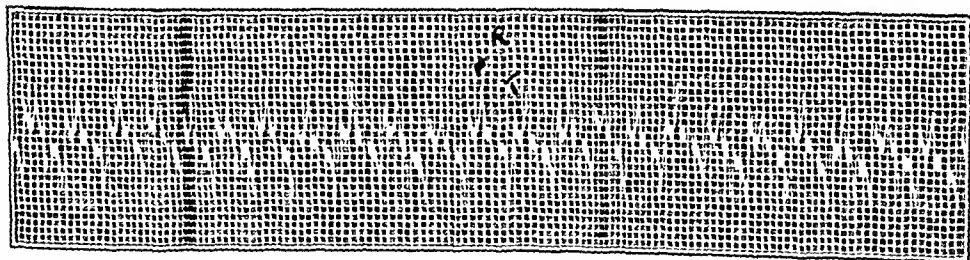


Fig. 2.—Electrocardiographic tracing, six hours after induction of peritonitis in an unprotected dog. The pulse rate is 264. The ST-interval is depressed. The T-wave is biphasic.

or one-half less (in mm. of mercury) than in the unprotected. There was an absence of arrhythmia which was observed in the unprotected dogs.

#### THE EFFECT OF PERITONEAL INFECTION ON THE HEART IN PROTECTED AND UNPROTECTED ANIMALS AS INTERPRETED FROM ELECTROCARDIOGRAPHIC TRACINGS

Electrocardiographic tracings were taken prior to and at half-hour intervals in unprotected dogs in which peritonitis was induced. The changes in the animals were progressive and rapid. There was a marked increase in pulse rate, from 90 to 220, in the space of four hours. Accompanying the rate increase was a depression of the ST-interval and the appearance of a biphasic T-wave. Six hours after the onset of peritonitis, the pulse rate had increased to 264, and a still greater T-wave deformity appeared. These changes were interpreted to

## THE CARDIOVASCULAR SYSTEM IN PROTECTED AND UNPROTECTED ANIMALS WITH ACUTE DIFFUSE PERITONITIS\*

BERNARD STEINBERG, M.D., AND J. LESTER KOBACKER, M.D., TOLEDO, OHIO

IN A PREVIOUS publication,<sup>1</sup> one of us produced experimental evidence that death in acute diffuse peritonitis is due to soluble toxic substances elaborated by bacteria present in the peritoneal cavity which are responsible for the peritonitis. This contention was based on experiments in which the peritoneal inflammation was induced by intraperitoneal injection of diphtheria bacilli suspended in gum tragacanth. Those animals which were given subcutaneous or intravenous diphtheria antitoxin invariably survived in spite of a marked peritonitis. Animals without antitoxin invariably succumbed.

Holzbach<sup>2</sup> in his review on circulatory failure in infectious conditions states that functional heart failure appears early in peritonitis, but this opinion is apparently based on clinical observation, since no experimental work on this condition was reviewed.

Two types of peritonitis were induced in dogs in our experiments: Fecal peritonitis by the intraperitoneal introduction of 5 gm. of small and large bowel feces suspended in 25 c.c. of saline; and, colon bacillus peritonitis by intraperitoneal injection of 50 c.c. of a 2 per cent gum tragacanth containing 200 million *B. coli* per c.c. Electrocardiographic and carotid pressure tracings on the kymograph before the onset and during the course of the peritonitis were the two criteria employed to determine the effect upon the heart. Two series of animals were employed: one series of twelve dogs in which no protection of any type was administered to the animals; in the other series of six animals all the dogs were protected by the administration intraperitoneally twenty-four to forty-eight hours before the onset of peritonitis of a suspension of killed colon bacilli in 1 per cent gum tragacanth (for the sake of brevity the material will be referred to as "coli-bactragen"). Previous publications<sup>3</sup> describe in detail the technic and the basis of this method of protection with coli-bactragen. It was desired to determine whether such a protection will evince any change which may otherwise be present in the unprotected animals on the electrocardiographic and kymographic tracings. All the animals were under pentobarbital sodium anesthesia. Along with each animal with peritonitis, a normal control dog was run simultaneously.

### THE EFFECT OF PERITONEAL INFECTION ON BLOOD PRESSURE IN PROTECTED AND UNPROTECTED ANIMALS

The effect on the blood pressure in the animals with fecal and *B. coli* peritonitis was alike. Within fifteen to twenty minutes after onset of the in-

\*From the Department for Medical Research, Toledo Hospital.  
Received for publication, February 27, 1935.

fection, the blood pressure began to drop gradually. In one hour there was a drop of 2 mm. of mercury. The fall was of the systolic pressure, the diastolic remained normal. The pulse pressure became progressively smaller. In six hours, the pressure dropped 5 mm. of Hg and at the same time an arrhythmia appeared. In the colon bacillus peritonitis, the fall was still more rapid although it seldom exceeded 4 mm. of mercury.

In the animals protected with coli-bactragen twenty-four to forty-eight hours prior to the onset of peritonitis, the systolic pressure dropped 1 mm. of mercury within ten to fifteen minutes and at the end of ten hours there was a total drop of 2 mm. of mercury. This drop in the protected animals was invariably a third

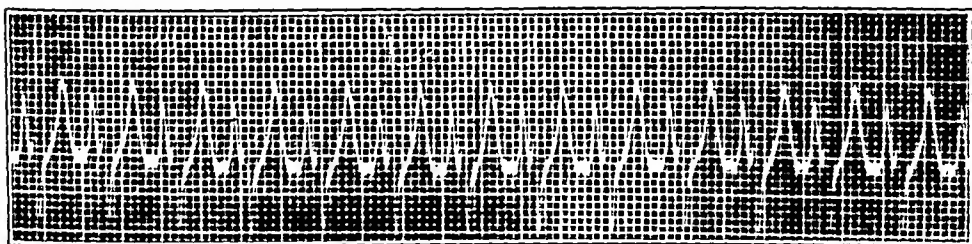


Fig. 1.—Electrocardiographic tracing, one-half hour after induction of peritonitis in an unprotected animal. The pulse rate is 200. (An increase from a normal of 90.) There is some depression of the ST-interval. In this as well as in all the subsequent figures Lead II is shown.

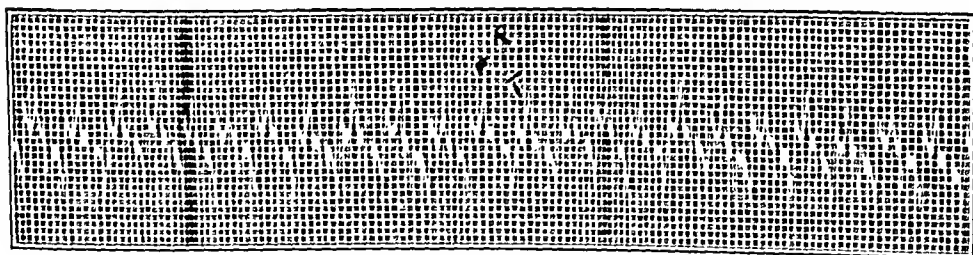


Fig. 2.—Electrocardiographic tracing, six hours after induction of peritonitis in an unprotected dog. The pulse rate is 264. The ST-interval is depressed. The T-wave is diphase.

or one-half less (in mm. of mercury) than in the unprotected. There was an absence of arrhythmia which was observed in the unprotected dogs.

#### THE EFFECT OF PERITONEAL INFECTION ON THE HEART IN PROTECTED AND UNPROTECTED ANIMALS AS INTERPRETED FROM ELECTROCARDIOGRAPHIC TRACINGS

Electrocardiographic tracings were taken prior to and at half-hour intervals in unprotected dogs in which peritonitis was induced. The changes in the animals were progressive and rapid. There was a marked increase in pulse rate, from 90 to 220, in the space of four hours. Accompanying the rate increase was a depression of the ST-interval and the appearance of a diphase T-wave. Six hours after the onset of peritonitis, the pulse rate had increased to 264, and a still greater T-wave deformity appeared. These changes were interpreted to



indicate myocardial damage. Irrespective of the type of peritonitis, whether fecal or colon bacillus, the changes were similar in character.

Six dogs were protected by the administration of coli-bactragen and forty-eight hours later peritonitis was induced. Three of the animals were injected with fecal material and the other three were given colon bacillus peritonitis. Shortly before the onset of the peritoneal infection electrocardiographic tracings

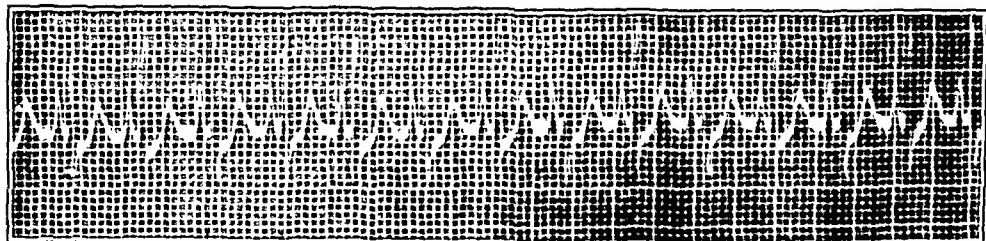


Fig. 3.—Electrocardiographic tracing forty-eight hours after introduction of coli-bactragen and before onset of peritonitis. The pulse rate is 180 (an increase from 100 before introduction of coli-bactragen). There is a slight depression of the ST-interval.

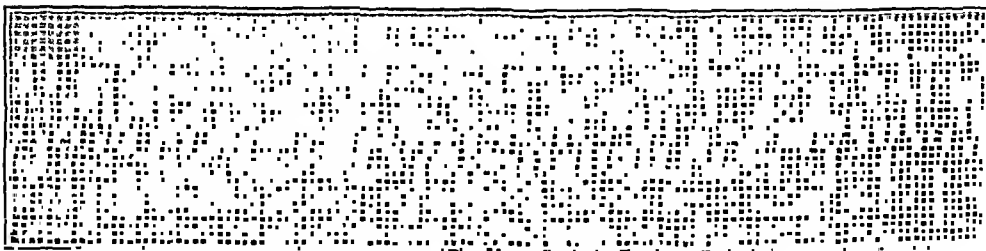


Fig. 4.—Electrocardiographic tracing two and one-half hours after onset of peritonitis in a protected dog. The pulse rate is 210. The complexes are normal.

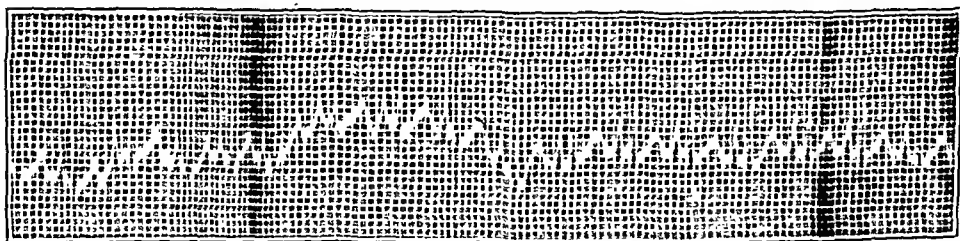


Fig. 5.—Electrocardiographic tracing six hours after onset of peritonitis in a protected dog. The pulse rate is 200. All the complexes are normal.

were taken (forty-eight hours after administration of coli-bactragen). The pulse rate was increased from 100 (before administration of coli-bactragen) to 180. The ST-interval was slightly depressed (Fig. 3). At half-hour intervals following induction of peritonitis, electrocardiographic tracings were made. The pulse rate increased to 210 in two and one-half hours. In six hours the rate was 200. In none of the tracings were there any changes in any of the ventricular or after-complexes which would indicate myocardial damage (Figs. 4 and 5).

## DISCUSSION

There are several conceptions regarding the type of organ or tissue upon which bacterial toxic substances elaborated in the peritoneal cavity exert their deleterious action. Manenkow<sup>4</sup> contended that the toxins ascended along the nerve lymphatics and paralyzed the medullary vascular centers. Kirschner<sup>5</sup> suggested that the capillaries of the peritoneum were paralyzed producing stagnation of the portal circulation. Askanazy<sup>6</sup> believed that the toxins dilated the intestinal lymphatics which pressed on the ganglion cells with a consequent paralysis of the bowels. No sufficient convincing experimental evidence has been brought forward to justify any of the above conceptions, although all the suggested tissues and organs may very well be acted upon at the same time by the toxic substances. Death may not necessarily be due to the action upon a single organ or tissue.

Some experimental evidence is presented in this work that indicates the involvement of the heart in acute diffuse peritonitis. The findings, under the conditions of our experiments, do not differentiate, however, myocardial damage due to direct action of the toxic substances from an ischemia which may result from a paralysis of peritoneal capillaries and diminished blood supply to the heart. The fall in the systolic pressure, the rapidity of the heart action, the changes in the ventricular and after-complexes in the electrocardiographic tracings may be interpreted either as a direct action of the toxin on the myocardium or as an indirect effect.

Animals which were protected against the lethal outcome in peritonitis by the administration of coli-bactragen failed to show any deleterious effects on the heart as determined by our criteria. The essential protective factor operating in animals with coli-bactragen consists of a rapid phagocytosis of the invading organisms. In previous articles<sup>1, 7</sup> it was demonstrated that successful protection of an animal depends on a rapid phagocytosis by a sufficiently large number of polymorphonuclears to cope with all the invading bacteria. This phagocytosis occurs within a few hours after the onset of peritonitis and consequently the bacteria are prevented from elaborating soluble toxic substances. This contention in former publications is further verified by experiments presented in this work so far as the effect upon the heart was not apparent in the protected animals.

## CONCLUSIONS

Under the conditions of these experiments, myocardial damage was demonstrated in acute diffuse peritonitis with carotid blood pressure and electrocardiographic tracings as criteria.

Animals protected against a lethal outcome from acute diffuse peritonitis by the administration of coli-bactragen, failed to reveal any myocardial damage.

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# LABORATORY METHODS

## OBSERVATIONS ON INTENSIFYING THE METACHROMATIC PROPERTIES OF CRESYLECHT VIOLET\*

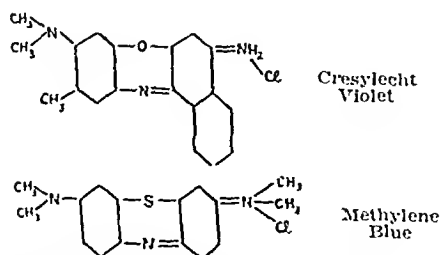
B. G. R. WILLIAMS, M.D., PARIS, ILL.

AN INVESTIGATION of the chemistry, physical properties and reactions of the cresyl violets was undertaken in 1922 at the request of the Commission on Standardization of Biological Stains. Several reports have been published.<sup>1-4</sup>

During the early part of 1934, it was decided to change somewhat the methods of study. Up to this time, it had not been regarded as practicable to intensify the metachromasia in a manner similar to that used with methylene blue: this for two very good reasons: (1) it already possesses fairly good metachromatic properties, and (2) it is very susceptible to alkalis even in traces.

Therefore as might be imagined, our first attempts to follow the methylene blue methods were very disappointing. But just as we were about to abandon the venture as one without promise, one experiment showed a very dramatic result. Finally last July a new and improved method for preparing a staining solution was elaborated. During the past six months this has been used routinely in surgical pathology. We have become sufficiently enthusiastic to publish the results, although, of course, further improvements will probably be made.

Contrary to expectations, it has been learned that alkalinization is practicable. However considerable pains must be taken when attempting to intensify: certainly much more care is needed than in the coarse methods used with methylene blue. In the first place, it seems imperative to use a good dye obtained from a constant source, for it has been pointed out<sup>1</sup> that the products of different makers vary greatly in properties. Moreover, as contrasted with the stable methylene blue, cresylecht violet is more unbalanced, delicate, and "fickle." This is observed not only when working with it, but is also suggested by its formula:



\*From the Central Office of Williams Pathological Service.  
Received for publication, January 22, 1935.

At any rate, it was decided early in the investigation to avoid heatings. Moreover no matter how hopeless a product might be secured, every effort was made to reclaim it. Chiefly upon these policies success was realized.

In all our experiments the item prepared by the National Aniline and Chemical Company was employed. We have been told that certain men still prefer to use imported cresyl violets. However it has been our experience when studying these foreign makes that no two of them agree in properties; and in our work, an item of constant and dependable composition is imperative.

#### PREPARATION OF THE SOLUTION

The several steps (as used at present) may be detailed as follows:

First, in a 250 ml. Florence Flask, mix well the following:

Cresylecht violet (National)	1 gm.
Potassium carbonate (dry)	1 gm.
White-water formaldehyde (U.S.P.)	5 ml.
Distilled water	95 ml.

Second, regardless of the physical appearance of the mixture, proceed with the work. Shake at intervals for about one-half hour.

Third, then carefully and in small amounts, add a total of 3 ml. glacial acetic acid. Gas is formed. Contents should be "swirled," or gently shaken in the flask, during the addition of the acid; but the flask should not be corked.

Fourth, swirl occasionally during the next one-half hour and then filter. Add 5 ml. alcohol, propyl, -iso (99 per cent) (Mallinckrodt).

The above first-step yields a brownish-muddy mixture, and it may be felt that, "all is lost." However the addition of the acid brings out a new, intense and beautiful violet. The exact changes we have not been able to determine. For want of a better name, we have been calling the new polychrome "cresyl orchid." However it is probably a composite or mixture rather than a definite substance; and probably we have merely (as with azures in polychrome methylene blue) added traces of minor components to the cresyl violet.

If the alcohol be omitted entirely, the solution will become weaker after a few days, and a reddish substance will be observed clinging to the glass. But prepared with the trace of alcohol, the solution seems to be stable for months. Here where much of it is used, we secure good results by preparing it fresh each month.

Reading over the above description, workers will recognize the rough skeleton of the Goodpasture method for polychrome methylene blue. However no heating operations are employed, the diluents are different, etc.

#### USE OF THE SOLUTION

The stain is of use in surgical pathology. It is applicable for rapid reading of tissues where prompt diagnoses are urgent. It quickly distinguishes between muscle and fibrous tissues without using elaborate methods like Van Gieson stain, etc. It may be employed with unfixed tissues, but gives excellent results where formaldehyde preparation has been used. It is particularly suited to work which must be rapid and yet accurate (biopsies, etc.) We have

not been able to mount these stains, and their value lies in diagnosis rather than permanent files. The following technic has yielded best results:

First, spread the section on the face of lifter.

Second, drain and gently blot off excess of water, leaving section just moist.

Third, with dropper or pipette, add a drop of the solution or just enough to cover the section.

Fourth, count off about six seconds. Then paddle off the section into a large dish of tap water. (We use a large Pyrex glass biscuit pan for this purpose.) After paddling it around for several seconds (rapid washing), place on a slide in water and add a cover glass.

#### THE METACHROMASIA

We have been taken to task for our former descriptions of granule staining with cresyl violets. It has been interesting to note, however, that there has been considerable disagreement among the critics. Very likely some of them have been using imported dyes. As a matter of fact, surgical pathologists are not greatly interested in granule staining. Therefore in this report we may wisely ignore the granules and describe the staining reaction of the important elements: *Blue*: all nuclei, muscle cytoplasm; *Pink*: other cytoplasm, fibrils; and *Yellow*: fat cells.

It is rather difficult to imagine a more valuable metachromasia in routine tissue work. Perhaps the stain falls short of expectations in autopsy work and where permanent mounts are wished; but for rapid surgical diagnoses, it is of great promise.

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# THE TECHNIC FOR ISOLATION AND PREPARATION OF BACTERIOPHAGE\*

GLADYS MORTON, B.S., AND MARIE WASSEEN, NEW YORK, N. Y.

**INTRODUCTION.**—Because of numerous requests for information concerning the practical laboratory methods of preparing bacteriophage, we will endeavor to present those used in this institution, discussing in turn, equipment and materials employed, sources and manner of isolation, and preparation of stock and specific bacteriophages.

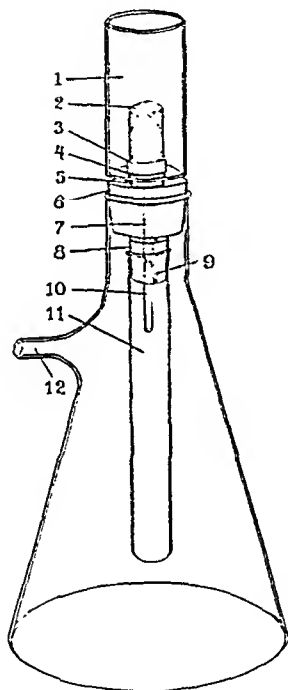


Fig. 1.—Assembled Berkefeld filter. (1) Mantle; (2) candle; (3) metal collar; (4) rubber washer; (5) metal nut; (6) rubber stopper No. 7, with single hole; (7) metal stem of filter candle; (8) rubber stopper No. 1, with two holes; (9) outlet hole through rubber stopper; (10) glass tube in continuity with metal stem; (11) test tube to receive filtrate; (12) side arm of filter flask to connect with suction.

**Equipment and Materials.**—Among the materials peculiar to this branch of work are filters. After trying various bacteriologic filters, the Berkefeld 3 W and 5 W have been found the most practical. The 5 W filter candles are used for filtering individual set-ups, and the 3 W for the larger volumes. The filters are assembled according to the diagram (Fig. 1). The test tube containing the filtrate may be replaced by an empty sterile tube and the filter used two or

\*From the Department of Pathology and Bacteriology, New York Post-Graduate Medical School and Hospital, Columbia University.

Received for publication, February 9, 1935.

These studies have been aided by a grant from the Josiah Macy, Jr., Foundation.

three times for succeeding generations if the material to be filtered is not too cloudy. Before assembling, new filters are brushed in running tap water and boiled for twenty minutes in distilled water. After using, the parts are boiled separately for twenty minutes in tap water. The mantles are washed and rinsed. The candles are brushed in running water to remove deposits on the outside, and then washed through with suction to remove all traces of medium which have collected throughout the pores, and are finally reboiled for twenty minutes. The parts are allowed to dry and the filters reassembled and autoclaved.

*Media.*—Two media are commonly used for all the bacteriophage work. The one used for testing and for making bacteriophage to be used locally is meat infusion broth. For subcutaneous and intravenous use, or in case of protein allergy, bacteriophage prepared in a comparatively protein-free medium in which the nutrient is asparagin, one of the amino acid group, is employed.

The asparagin medium<sup>1</sup> is prepared as follows:

Asparagin	3 gm.
Magnesium sulphate	2 gm.
Sodium chloride	4.5 gm.
Bipotassium hydrogen phosphate	2 gm.
Distilled water (neutral in reaction)	1,000 c.c.

Dissolve the substances in water. Bring to a boil, adjust the pH to 7.6. Autoclave at 15 pounds for fifteen minutes. Filter through filter paper, tube, and autoclave again at 15 pounds for thirty minutes. Final pH should be 7.0 to 7.2. A precipitate is usually formed during the first autoclaving. The medium should be clear after the second autoclaving.

*Isolation of Bacteriophage.*—In isolating a bacteriophage different sources may be utilized. Sewage is the most common source and a bacteriophage for the colon bacillus can almost invariably be obtained. The sewage is filtered through a Berkefeld filter and set up as indicated in Table I.

TABLE I

	ORGANISM CONTROL C.O.	ORGANISM AND SEWAGE FILTRATE		SEWAGE FILTRATE STERILITY TEST C.O.
		4 HOURS C.O.	24 HOURS C.C.	
Meat infusion broth	10.0	10.0	10.0	10.0
Filtered sewage	—	0.5-1.0	0.5-1.0	0.5-1.0
Broth suspension of 24-hour culture	0.1	0.1	0.1	—

The suspension should be heavy enough to make a barely perceptible cloud when 0.1 c.c. is inoculated into 10 c.c. of broth. The tubes are then incubated at 35° C. until there is a perceptible growth in the control, usually about four hours. At that time the preliminary reading is made. An arbitrary scale of clearing has been adopted as follows:

Four-plus lysis	Complete clearing
Three-plus lysis	Slightly cloudy, much less growth than in the control, and with no bacterial sediment
Two-plus lysis	Perceptible clearing, but with some sediment
One-plus lysis	Heavy growth, only slightly clearer than the control.



At this time one tube is filtered, the filtrate placed in the ice box, and the other tubes remain in the incubator overnight. A reading is then made, using the same scale. If the lysis is not four-plus at this time the process is repeated, using the filtrate which has been procured after the four-hour reading. When lysis is complete, a volume of the bacteriophage is prepared, using the same materials and amounts as described above, but setting up a greater number of tubes so that a larger amount can be obtained. It is then titrated by the fluid method described by d'Herelle.<sup>2</sup>

Other sources from which bacteriophage may be isolated include specimens from patients in either chronic, acute or convalescent stages of disease; sludge, animal feces, and impure water. Slight variations in the method of isolating bacteriophage may be employed. The sample from which a bacteriophage is desired may be inoculated with a susceptible strain of organism and incubated twenty-four or forty-eight hours before the first filtration. In isolating bacteriophage from patients' specimens, such as pus, blood or feces, the specimen is placed in nutrient broth and incubated overnight before it is filtered.

The potency of a bacteriophage is ascertained by titration (d'Herelle<sup>2</sup>). A titration of  $10^8$  is required for all stock races, and a higher titer is desirable whenever possible. The titer may be raised in some cases by serial filtration even after four-plus lysis for twenty-four hours has been reached.

*Testing of Specimens.*—The organisms which are to be tested for bacteriophage susceptibility are isolated and transplanted on agar slants, or where the growth is scant, in broth. Stock bacteriophage is used as the filtrate and the organism is set up and serial filtration carried out by the same method as is used in isolating a bacteriophage (see Table I).

The process is repeated until the lysis is complete, or for five generations, after which, if there is no sign of lysis, or no noticeable improvement, the culture is reported not susceptible or partially susceptible as the case may be. It is important to remember that not all organisms are susceptible to bacteriophage.

If an asparagin bacteriophage is desired, the suspension is made in asparagin, and not more than 0.5 c.c. of broth filtrate is used for 10 c.c. of asparagin medium. Where the bacteriophage is to be used for intravenous injections, it is advisable to carry it for two generations in asparagin, thus reducing the broth concentration to a negligible minimum.

Volumes of bacteriophage specific for the organism are prepared as soon as four-plus lysis, which persists for twenty-four hours, has been obtained. This is done by setting up several tubes with the organisms and the filtrate which gives the four-plus lysis. After four hours this is filtered and sterility tests are made by placing 1 c.c. of the bacteriophage in a tube of nutrient broth and incubating forty-eight hours. If no growth is visible in that time it is assumed that the filtrate is sterile. No bacteriophage which shows the slightest cloud should be used in therapy.

Aseptic precaution must be observed in tubing the bacteriophage, since any air contamination would render it unfit for use. A special room in which all draft is eliminated and which can be washed with an antiseptic is utilized.

The bacteriophage is tubed in amounts applicable for use in the particular case, since after opening the tube it is advisable to use the entire contents. For subcutaneous and intravenous injections the bacteriophage is put up in a vaccine vial with a soft rubber cap.

When a bacteriophage is made up with an organism isolated from a patient's culture it is called "specific" to differentiate it from stock preparations. It is advisable to use this specific bacteriophage whenever possible since it contains, in addition to the lytic principle, the dissolved substance of the invading organism of the patient. Stock bacteriophage is recommended, however, in acute cases, while the specific is being prepared.

*Preparation of Stock Bacteriophage.*—Stock bacteriophage has a twofold purpose: the testing of organisms and the therapeutic use until a specific preparation is available. It is therefore necessary that it be polyvalent and that it have a high titer. Methods vary in obtaining these qualities for different groups of bacteriophage.

*A. Staphylococcus:* In this laboratory staphylococcus bacteriophage is most widely used. It embodies seven individual races isolated from different sources.\* A combination of these phage races in equal parts is set up with a number of virulent and recently isolated staphylococcus strains and filtered together. The new stock is always titrated with one of the strains used in its preparation.

*B. Coli:* Two methods have been found useful in preparing a polyvalent stock coli preparation: first, increasing the number of individual stock races; second, the addition of specific bacteriophages which have been prepared for case strains. The stock coli mixture used in testing and in therapy is a combination in equal parts of forty individual races.† This mixture has been set up with *Coli C.*, the susceptible strain used for the original isolation, in addition to a number of susceptible case strains. Before the mixture is filtered, 10 or 15 c.c. of the specific bacteriophages for each recent case strain are added. Every six months, or more often if the original mixture has become too specific, a new one is made from the original races.

*C. Streptococcus:* Streptococcus bacteriophage is highly specific and four-plus lysis is obtained in but a few of the strains tested. The stock which is used for testing is a combination of six individual races,‡ prepared with a hemolytic and a fecal strain. Since it is not possible to prepare fully potent specific bacteriophage for a majority of the case strains, a partially potent one is often used and has been found of practical use in therapy.

The set-up in Table II is made in duplicate, and one set-up, with exception of the organism control and bacteriophage sterility, is filtered as soon as there is sufficient growth for reading. The duplicate is left in the incubator for a twenty-four-hour reading. The filtrate is then set up in the same manner and

\*Staphylococcus races of bacteriophage have been obtained from Bronislawa Fejgin and A. Gratta.

†Coli races of bacteriophage have been obtained from B. R. Callow, Julius Klosterman, Ralph S. Muckenfuss and Gregory Schwartzmann.

‡Streptococcus races of bacteriophage have been obtained from F. d'Herelle, Alice Evans (race isolated by P. F. and A. S. Clark), Norman R. Goldsmith, Helen Jern and Gregory Schwartzmann.

the tubes in which complete lysis occurs are filtered and used in therapy. The remaining tubes are filtered and again set up in the same dilutions to see if a fully potent bacteriophage can be obtained.

*D. Miscellaneous:* The miscellaneous group embodies single races of pyocyaneus, proteus, typhoid, Flexner and Shiga bacteriophages which are prepared with the strains originally used in isolation. Volumes are prepared by using the routine method.

TABLE II

	ORGANISM CONTROL	BACTERIAL SUSPENSION + BACTERIOPHAGE						BACTERIOPHAGE CONTROL
	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.	C.C.
Meat infusion broth	10.0	—	2.0	6.0	8.0	9.0	9.5	9.5
Bacterial suspension	0.1	0.1	0.1	0.1	0.1	0.1	0.1	—
Bacteriophage	—	10.0	8.0	4.0	2.0	1.0	0.5	0.5

*Mixed Bacteriophage.*—In cases where a mixed bacterial infection is involved, the bacteriophages are mixed in proportions as indicated by the specific culture. In preparing stock intestinal mixture 100 colitis cases were studied before deciding the appropriate proportion. At the present time it contains about 48 per cent mixed coli bacteriophage, 24 per cent streptococcus bacteriophage prepared with a fecal strain, 24 per cent prepared with a hemolytic strain, and 4 per cent staphylococcus bacteriophage.

No preservative is used in bacteriophage, since the amount necessary to inhibit the growth of bacteria also inhibits the bacteriophage. Aseptic precaution and careful sterility tests are employed. The bacteriophage is kept in the refrigerator and should maintain its potency from four months to three years in this condition. Bacteriophage is sensitive to high temperatures and in incubating the temperature should not be over 35° C. in order to get the best results. Some bacteriophages fail to cause complete lysis if the incubator temperature is increased.

We deem it a privilege to acknowledge the helpful guidance of Professor Ward J. MacNeal and Doctor Adele E. Sheplar, Chief of the Laboratory of Bacteriology, and also the collaboration of our associates in this laboratory.

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## PREVENTING AIR HOLES IN SERUM CULTURE MEDIA\*

SISTER M. EDELTRUDE PETE, ST. PAUL, MINN.

THE difficulty of producing Loeffler's blood serum culture media without air holes has been a problem to me for sometime, and I discovered it to be such to many others. As a consequence I experimented, and, having found an easy method of offsetting the air hole disturbance, I am offering it to those whom it may interest.

We who have worked in laboratories are well aware that Loeffler's blood serum culture media, as routine media for common culture, have been used with great success, but considerable expense when purchased ready for use. We know also, that to produce these media without bubbles and for long preservation of moisture, is a problem. A little knowledge of the cold-pack and steam-pressure method of canning brought this idea to me; why not use that procedure for culture media?

I mixed the culture media according to the direction given for the Loeffler's blood serum, and put it into small vials or bottles (17 by 60 mm.), using for plugs, corks to about one-sixteenth of an inch from the top of the bottle, and screwing over them tightly fitting aluminum cork-lined caps; and on some others I screwed the aluminum cork-lined caps without the corks. I placed both kinds of vials into the autoclave in a slanting position following the directions for the coagulation and sterilization. Upon removal from the autoclave I found the vials entirely free from air holes or bubbles, the media sufficiently thick and with a smooth surface, plus sufficient water condensation in the bottles to assure indefinite moisture while the bottles were kept in a cool place.

While the cork of a vial remains tight in the autoclave, the expansion of the media during the heating process will cause the rising air to remain in a layer just below the cork, thus preventing bubbles, while the pressure from without keeps the bottles from breaking. If, however, this rising air in the bottle, under the pressure of heat, is allowed to escape from the bottle, the change of pressure within the vial will cause air holes in the media, and sometimes breakage of bottles.

The culture media prepared from Loeffler's blood serum with the aluminum cork-lined caps, which I have described, are so much less expensive than what we had in use, that we are using the former freely now for all of our common routine culture media.

These vials, cork-lined aluminum screw caps, 17 by 60 mm., are \$0.35 per dozen, or \$3.50 per gross, and can be obtained from the Arthur Thomas Company, Philadelphia.

\*From St. Joseph's Hospital.

Received for publication, February 15, 1933.

## BAGG ALBINO MICE FOR TESTING HORMONES AND OTHER EXPENSIVE SUBSTANCES\*

J. F. McCLENDON, PH.D., AND HAROLD STREET, M.S., MINNEAPOLIS, MINN.

MICE have been used both by American and European workers in assaying theelin and other substances. The use of mice is particularly advantageous when the amount of hormone in the blood is to be assayed, and yet many workers are prejudiced against the use of mice. They claim mice are delicate, and for nutrition studies the growth rate is too slow. On the other hand, I have found mice quite hardy, standing travel for many thousands of miles in winter weather and living in cold rooms. This is especially true of wild mice who live in unheated shacks with an outside temperature of 30° below zero. I have found the mice that were delicate were infected with some epidemic. Hot weather, however, is more detrimental and care must be taken that they have water at all times and, though very little oxygen is needed, a slight amount of ventilation is necessary for evaporation of moisture in heat regulation.

Bagg albino mice have been used by a number of workers, and it seems to be the most homozygous strain obtainable. From the work of Fortuyn on the albino mouse, and Palmer and his students on rats, it would seem that in selecting for homozygosity, it may be necessary to pay special attention to the particular character whose variability it is desired to reduce.

I have bred Bagg albinos for a number of years and established colonies not only in Minneapolis but also in Sendai and Sapporo, Japan. They grew well in rooms at 40° F. when the metal cages were surrounded by thin paper. They grew well on diets used for rats and developed rickets on Steenbock's diet No. 2965. Harold Street and I have determined their variability in body weight on the following diet:

Ground whole wheat	40.0%
Ground whole flax seed	4.5
Whole milk powder	45.0
Iodized salt	2.2
Yeast	3.3
Alfalfa dust	5.0

as given in Table I, in which 30 to 50 mice of each age and sex were weighed. The coefficient of variability is slightly greater than that observed by King in Wistar albino rats (about the same as in Norway rats) but is not sufficiently greater to warrant the use of rats for very rare substances. Even when the growth rate is taken as an index of physiologic test, it is not the amount of growth which determines their worth but their variability. It would be desirable for

\*From the Laboratory of Physiological Chemistry, University of Minnesota.  
Received for publication, February 27, 1935.

TABLE I

BAGG ALBINO MICE: COEFFICIENTS OF VARIABILITY; MEANS AND PROBABLE ERRORS OF MEANS IN GRAMS

DAY OF AGE	MALES			FEMALES		
	MEAN WEIGHT	COEF. VAR. %	PROBABLE ERROR OF MEANS	MEAN WEIGHT	COEF. VAR. %	PROBABLE ERROR OF MEANS
1	1.31	15	0.0246	1.30	15	0.0228
2	1.51	16	0.0306	1.52	13	0.0231
3	1.75	16	0.0314	1.77	18	0.0317
4	2.09	19	0.0409	2.16	15	0.0317
5	2.45	19	0.0501	2.57	17	0.0449
6	2.88	19	0.0598	3.02	18	0.0567
7	3.30	17	0.0631	3.47	23	0.0805
8	3.77	18	0.0736	3.97	19	0.0784
9	4.21	18	0.0841	4.43	20	0.0921
10	4.67	17	0.0843	4.92	20	0.1006
11	5.02	19	0.1038	5.32	21	0.1138
12	5.39	21	0.1236	5.63	23	0.1327
13	5.78	20	0.1288	6.04	22	0.1354
14	6.10	19	0.1272	6.48	22	0.1438
15	6.41	19	0.1315	6.64	23	0.1562
16	6.57	16	0.1164	6.92	21	0.1521
17	6.78	17	0.1294	7.04	22	0.1596
18	6.85	22	0.1645	7.17	21	0.1562
19	6.96	21	0.1621	7.24	23	0.1684
20	7.13	21	0.1605	7.41	23	0.1702
21	7.33	20	0.1676	7.72	21	0.1741
22	7.67	21	0.1812	7.95	22	0.1856
29	9.48	19	0.2601	9.87	19	0.2050
36	12.36	19	0.2601	12.50	18	0.2477
43	14.74	15	0.2588	14.47	15	0.2532
50	16.74	15	0.2860	15.61	15	0.2581
57	18.54	16	0.3496	16.64	14	0.2739

TABLE II

ALBINO MICE, MEAN WEIGHT IN GRAMS

AGE	ROBERTSON 1916	ROBERTSON AND CUTLER 1916	ROBERTSON AND DELPRAT 1917	FORUYN WM	FORUYN HTM	GATES MALES	GATES FEMALES	MC CLENDON AND STREET MALES	MC CLENDON AND STREET FEMALES
Birth	1.23	1.47	1.47	1.31	1.41	1.38	1.38	1.31	1.3
1 wk.	3.31	2.99	3.35	3.48	3.97			3.77	3.97
2 wk.	5.14	5.09	4.44	6.03	6.24	5.49	5.64	6.41	6.69
3 wk.			5.89	8.65	8.74			7.67	7.95
4 wk.			8.55	11.64	12.07			9.48	9.87

purposes of correlation of the work of different investigators if more workers on mice used the same breed just as has been done in the case of the Wistar rat.

Table II shows the variation one obtains in using different breeds of mice.

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## A CONVENIENT APPARATUS FOR RAPID AND ACCURATE TUBING OF CULTURE MEDIA\*

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ELIZABETH S. PORTER, M.S., AND RALPH MCBURNEY, M.D., UNIVERSITY, ALA.

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THE simplicity and convenience of this device are its chief recommendations, especially where large quantities of media are to be quickly tubed. It enables one to fill a basket of tubes to the same approximate level without moving the tubes, and with more convenience than by holding three or four tubes in the hand and transferring them from one basket to another. Uncomfortable handling when tubing hot agar is eliminated.

Any carpenter can make the wooden structure which may then be finished in dark oak or black. Necessary parts may be used from laboratory stock.

A general view of the boxlike structure is shown in Fig. 1. Detailed features of construction are shown in Fig. 2.

The wooden structure is essentially a box. It is 10 inches wide and  $9\frac{1}{4}$  inches deep at the base. At a height of 7 inches the frame is "stepped" back  $4\frac{1}{2}$  inches, on which level is fastened a  $\frac{3}{4}$  inch board, 11 inches long, and  $4\frac{3}{4}$  inches wide, forming a shelflike stage upon which a wire basket of tubes is placed during the tubing of media. Each side is  $4\frac{3}{4}$  inches deep above this level, rising to a height of 9 inches, making the back 16 inches high. It is topped with a  $\frac{3}{4}$  inch board similar to that used in the construction of the stage. It is held in place by small hooks and screw eyes on each side, allowing easy removal for replacing the electric light bulb used for illumination and described later.

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\*From the Department of Bacteriology, School of Medicine, University of Alabama.  
Received for publication, February 23, 1935.

In the front, level with the top of the stage, is a window  $5\frac{1}{2}$  inches wide and  $6\frac{1}{2}$  inches high. It is fitted with ground glass held by  $\frac{7}{16}$  inch moulding. The whole is recessed to be in the same plane with the sides.

Inside the box, behind the window, is a movable shade which is raised or lowered by a set screw (*a*) (Fig. 2). The shade is made of a piece of sheet tin or aluminum 3 by  $6\frac{1}{2}$  inches and suspended by two pieces of wrapping twine tied to a hole punched in each end of the sheet metal, the free ends being knotted through  $\frac{1}{8}$  inch holes drilled in a  $\frac{3}{8}$  inch brass crossbar. The holes in the bar are spaced so the strings will hang parallel. The brass bar is made of hollow tubing and is held in place by pushing through holes drilled in each side,

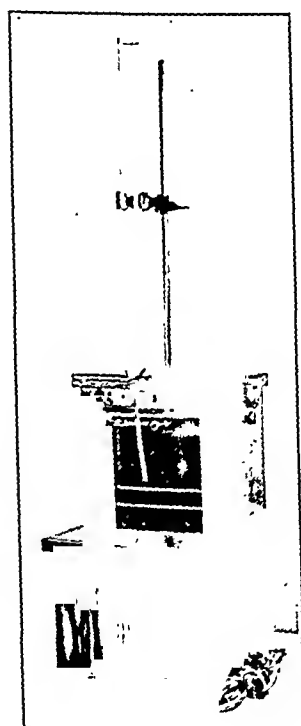
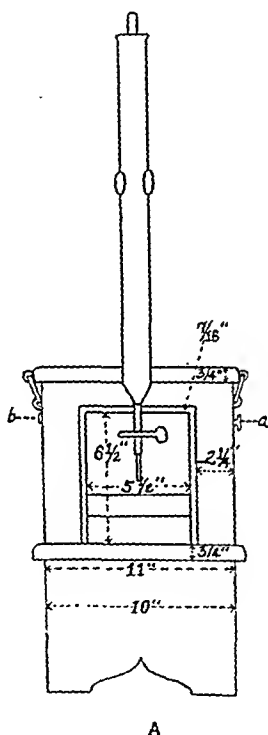
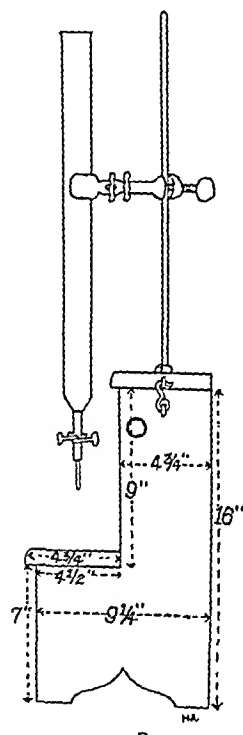


Fig. 1.



A



B

Fig. 2.

about  $\frac{3}{4}$  inch from the front and just above the level of the ground glass window. The holes should be a little larger than the crossbar to allow easy turning. The set screw (*a*) (Fig. 2), used for raising and lowering the curtain, is threaded onto the outside of the bar. At *b* the bar is held by a small metal washer with a machine screw threaded into the tubing.

To prevent the metal curtain from swaying backward a small piece of copper or iron wire is stretched from side to side behind it, about two inches below and in the same plane with the metal crossbar.

A porcelain electric light socket is fastened vertically to a small wooden strip on the inside of the box extending from side to side and approximately level with the height of the stage. Ordinary electric lamp cord is connected to this through the bottom and coming out at either side underneath. A hand



switch may be placed between this and a connection for plugging into any light socket. A 40-watt frosted bulb is ample for illumination. The interior of the box is painted white.

Fastened vertically into the center of the top is an 18 by  $\frac{3}{8}$  inch iron rod. It is fastened by a nut on both sides of the board with iron washers between and loose enough to allow for free swing of the buret attached by an iron clamp as shown in Fig. 2.

The buret into which media are poured, for tubing, may be adjusted to a convenient height by means of the iron clamp. The buret used in this instance has a capacity of 250 ml. It may be removed for washing, and may be wrapped in paper and sterilized, if necessary for any special media.

Rubber tubing with a glass tip is attached to the end of the buret, for distributing media to the tubes. This rubber tubing should be long enough to reach each tube comfortably. It is fitted with a pinch-cock clamp to control the flow of media.

On the window are two horizontal lines to indicate the levels to which tubes are to be filled for broth cultures, agar slants, and pours. The lines may be drawn with an ordinary blue grease pencil used for marking glassware. The operator sits so that his eyes are on a level with one of these lines. Illumination is from the light inside the box. The ground glass of the window and the shade, which is adjusted to eye comfort, prevent glare.

A basket filled with tubes is placed upon the stage. Tubes are filled in rows beginning at the back and working forward. This permits the operator to see the guide lines for filling.

With the free swing of the buret bar and the rubber tubing and height of the buret properly adjusted, no trouble will be experienced in conveniently reaching every tube.

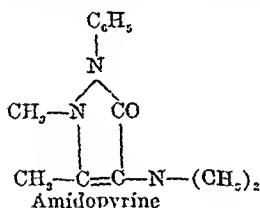
# A LABORATORY STUDY OF AMIDOPYRINE, BARBITAL, PHENYL HYDRAZINE, AND BENZENE IN RELATION TO AGRANULOCYTIC ANGINA\*

V. L. BOLTON, A.B., KANSAS CITY, KAN.

**A**GRANULOCYTIC angina was described by Schultz<sup>1</sup> in 1922, with the suggestion that an unknown chemical agent or microorganism might be the cause of the disease. Recent opinion points to an explanation involving the effects of chemical substances, represented by a number of drugs.

During the past twelve years administration of the benzene, pyrazolone and barbituric acid derivatives has been associated with the occurrence of the disease in man. Suggestive results also have been reported with reference to the experimental production of granulocytopenia in animals, by the use of amidopyrine, benzene, and the barbiturates.

The clinical evidence emphasizes amidopyrine as a possible etiologic factor in the production of agranulocytic angina. Amidopyrine is dimethyl-amino-phenyl-dimethyl-pyrazolone, a compound containing phenylhydrazine, imino groups, pyrazolone, and the benzene nucleus as potentially toxic fractions.



In the following experiments are reported the effects of amidopyrine, barbitol, amidopyrine and barbitol, phenylhydrazine, and benzene on the cellular elements of the blood of normal dogs. Both the total cell count and differentials were done on venous blood. The results were checked by Miss Esther Rodewald, technician in the hospital laboratory.

## RESULTS

### *Experiment I.—Small doses of amidopyrine.*

Dog: Weight 10 kg. Total white count 13,000; with 80 per cent neutrophils, 16 per cent lymphocytes, 2 per cent monocytes, 1 per cent basophils, and 1 per cent myelocytes.

June 18: 0.3 gm. of amidopyrine, in water, was administered by stomach tube each day for twenty-one days, a total of 6.3 gm. The white count increased to a range of 18,000 to 20,000; it remained elevated with no significant change in the differential.

July 9: 3 gm. was introduced by stomach tube.

July 10: 0.5 gm., in 5 per cent solution, was injected hypodermically.

July 11: Dog died; 9.8 gm. of amidopyrine had been given, about 1 gm. for each kilogram, in a period of twenty-three days.

\*From the Department of Pharmacology, University of Kansas, School of Medicine.  
Received for publication, February 13, 1935.

*Necropsy:* Sections of the bone marrow of the femur, humerus, and ribs, of the liver, kidney, and spleen were made. There was hyperplasia of the bone marrow, with no decrease in the myeloid elements; some fatty change in the liver; interstitial infiltration of mononuclear cells in the kidneys; congestion of the spleen, with increase of red cells in the sinusoids and prominence of the malpighian bodies.

*Experiment II.*—Large doses of amidopyrine.

Dog: Weight 10 kg. Total white count 12,200; with 86 per cent neutrophiles, 12 per cent lymphocytes, 1 per cent eosinophiles, 1 per cent myelocytes.

June 18: 1 gm. of amidopyrine, in water, was given by stomach tube each day for seven days, a total of 7 gm.

June 25: 2 gm. were then given each day for nine days, a total of 18 gm.

July 3: 3 gm. were given each day for four days, a total of 12 gm. This represents 37 gm. of amidopyrine, orally; or 3.7 gm. for each kilogram in a period of twenty-five days.

July 7: differential count: 86 per cent neutrophiles, 8 per cent lymphocytes, 2 per cent basophiles, 2 per cent myelocytes, 1 per cent eosinophiles, 1 per cent monocytes. There was a considerable number of normoblasts in the film.

July 8: 8.3 gm. of amidopyrine, 5 per cent aqueous solution, in doses ranging from 0.4 gm. to 1 gm. daily, were given intravenously, in a period of thirteen days. During this interval the total white count began at 12,000; dropped to 6,650 on the third and fifth days of injection; then gradually returned to 12,000 on the thirteenth day of injection. No important change in the differential count was observed.

There had been given 45.3 gm. of amidopyrine, or 4.53 gm. for each kilogram over a period of thirty-three days.

*Experiment III.*—Sodium barbital.

Dog: Weight 10.5 kg. Total white count 15,000; with 88 per cent neutrophiles, 10 per cent lymphocytes, 2 per cent monocytes.

June 18: 0.5 gm. of sodium barbital, in water, was given by stomach tube each day for fifteen days, a total of 7.5 gm.

July 3: 1 gm. was administered each day for five days, a total of 5 gm. This represents 12.5 gm. of sodium barbital, or about 1.2 gm. for each kilogram, over a period of twenty days.

The minimum total white count was 11,000 on July 7; with 83 per cent neutrophiles, 10 per cent lymphocytes, 4 per cent myelocytes, 2 per cent basophiles.

*Experiment IV.*—Amidopyrine and sodium barbital.

Dog: Weight 9 kg. Total white count 12,000; with 83 per cent neutrophiles, 13 per cent lymphocytes, 2 per cent monocytes, and 2 per cent myelocytes.

June 18: 0.3 gm. of amidopyrine and 0.5 gm. of sodium barbital, in water, were given by stomach tube each day for fifteen days; a total of 4.5 gm. of amidopyrine, and 7.5 gm. of sodium barbital. The white count increased to 18,150; with no appreciable rise in neutrophiles.

July 3: 1 gm. of amidopyrine and 0.5 gm. of sodium barbital were given each day for seven days, a total of 7 gm. of amidopyrine, and 3.5 gm. of sodium barbital.

July 10: 3 gm. of amidopyrine and 1.5 gm. of sodium barbital were given each day for seven days, a total of 21 gm. of amidopyrine and 10.5 gm. of sodium barbital.

July 18: 0.5 gm. of amidopyrine, 5 per cent solution, was injected hypodermically.

July 19: 0.5 gm. of amidopyrine, 5 per cent solution, was injected hypodermically.

July 20: 1 gm. of amidopyrine, 5 per cent solution, was injected hypodermically.

This represents 28.5 gm. of amidopyrine, 3.2 gm. for each kilogram; 21.5 gm. of sodium barbital, 2.4 gm. for each kilogram, in a period of thirty-two days.

The total white count decreased gradually to 10,000; then returned to 13,000. There was no important change in the differential count.

*Experiment V.*—Phenylhydrazine.

Dog: Weight 16.2 kg. Total white count 12,200; with 83 per cent neutrophiles, 14 per cent lymphocytes, 2 per cent myelocytes, 1 per cent basophiles. Red cell count 5,550,000; hemoglobin 84 per cent by the Haden-Hausser hemoglobinometer.

June 18: 0.3 gm. of phenylhydrazine hydrochloride, in water, was given by stomach tube each day for four days, a total of 1.2 gm. or 74 mg. for each kilogram in a period of four days.

June 21: Red cell count 4,190,000.

June 25: Red cell count 1,160,000; hemoglobin below 18 per cent.

June 26: A total white count made daily showed a gradual increase to 45,000; with 91 per cent neutrophiles.

July 3: The red cell count gradually increased to 4,930,000 and the total white count decreased to 12,000.

At no time was the granulocyte count below the control level.

*Experiment VI.—Benzene.*

Dog: Weight 17 kg. Total white count 13,000; with 81 per cent neutrophiles, 15 per cent lymphocytes, 4 per cent myelocytes. Red cell count 5,000,000; hemoglobin 84 per cent.

July 5: Benzene, 50 per cent in olive oil, was given in capsules in doses of 2 to 12 c.c. each day for thirteen days, a total of 80 c.c. of benzene or 4.7 c.c. for each kilogram.

July 13: White count 8,150; with 67 per cent neutrophiles, 24 per cent lymphocytes, 4 per cent myelocytes, 3 per cent eosinophiles, 2 per cent monocytes.

July 14: Red cell count 4,900,000.

July 20: Total white count 13,000.

#### DISCUSSION

In this series of experiments, definite leucopenia was observed only in those dogs which received large doses of amidopyrine, or of benzene. Absence of significant changes in the granulocyte ratio in the differential count makes it impossible to state that a blood picture comparable to that of granulocytopenia was produced in normal dogs. Attempts to reproduce the disease experimentally by Madison and Squier,<sup>2</sup> Hoffman, Butt and Hickey,<sup>3</sup> and by Kracke<sup>4</sup> have been partially successful.

The present experimental evidence and clinical observation on the relation of drugs to granulocytopenia require further theoretical analysis. This analysis must involve a discussion of the chemistry of amidopyrine and of the barbiturates in relation to their pharmacologic actions.

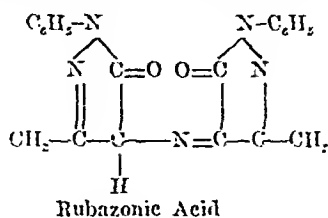
Amidopyrine is dimethyl-amino-phenyl-dimethyl-pyrazolone. Its synthetic precursor, antipyrine, is produced by the condensation of methyl-phenylhydrazine with acetoacetic ester; to antipyrine a dimethyl-amino group is added. There are, therefore, three possible toxic fractions in amidopyrine: pyrazolone, the nitrogen radicals, and methyl-phenylhydrazine. The benzene nucleus found in phenylhydrazine might also be considered injurious, but it should be emphasized that this nucleus is the phenyl group, which is unlike pure benzene.

The action of pyrazolone on the cellular elements of the blood is not described by Fränkel,<sup>5</sup> but its close relation to pyrrole, a toxic substance, may be significant.

Tertiary nitrogen, as found in amidopyrine, is the least toxic of the basic nitrogen radicals. It is potentially toxic in as much as reduction in the body may produce a true imino group. This group has been reported toxic by Herz,<sup>6</sup> but it is not necessarily toxic for myeloid elements.

Fränkel<sup>5</sup> states, according to Joffe, rubazonic acid appears in the urine after the use of amidopyrine.

Its structure indicates a demethylation of all the methylated nitrogen groups. Since McGuigan<sup>7</sup> states that following the use of amidopyrine, rubazonic acid occurs in the urine after standing, and produces a red color due to oxidation,

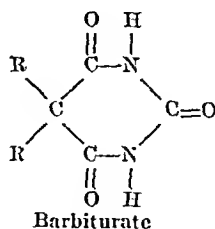


the formation of rubazonic acid in the body from amidopyrine is questionable. Amidopyrine is also excreted in the urine as a paired glycuronic acid, and as antipyril urea.

In spite of the toxic capacity of the imino group as such, there still remains the more serious possibility of blood destruction by methyl-phenylhydrazine as an hydrolytic cleavage product of amidopyrine.

It is generally accepted that the erythrocyte and granulocyte arise from a common stem cell, the hemoctyblast. In granulocytopenia there is anemia as well as leucopenia. In view of this fact, it would appear that the toxic action of phenylhydrazine, or an unrecognized modification, may in some people be extended to the stem cell or to an early form in the process of its maturation.

The importance of the barbiturates, as stated by Watkins,<sup>8</sup> has not been explained. Herz has suggested that the toxicity of amidopyrine is based on the NH radical, and that the attached benzene ring is insufficient to neutralize its toxicity. Recognizing the superior toxicity of NH over tertiary nitrogen, the barbiturates should be more toxic than amidopyrine, since two NH groups are found in the barbiturate ring, whereas no incompletely substituted nitrogen atoms are present in amidopyrine.



On the contrary, the prevalence of barbiturate medication as a hypothetical factor in the etiology of the disease is relatively slight in comparison with that of amidopyrine.

Benjamin and Biederman<sup>9</sup> feel that the disease is probably due to a drug hypersensitivity, with the blood system acting as the seat of the shock. Pepper<sup>10</sup> has also called attention to drug allergy in leucopenia. These hypotheses are supported by the fact that many drug hypersensitivities occur, in some of which the formed elements of the blood are affected.

In agranulocytic angina the blood changes are the most important findings. It is logical, therefore, to assume that hypersensitivity may not be the only

factor, since it is known that amidopyrine and the shorter acting barbiturates are altered in the body. These drugs contain fractions which may exert a toxic action in the process of their alteration.

It is apparent, then, that more than one type of reaction and numerous chemical substances could be involved in the etiology of agranulocytic angina. Experiments are in progress in this department to determine the nature of this reaction and of these chemical substances, with special attention directed to the effect on myeloid maturation.

#### SUMMARY

1. A study has been made of the effects of amidopyrine, barbital, phenylhydrazine, and benzene on the blood count in a series of six dogs.

2. A moderate leucopenia was observed in one animal which received amidopyrine, and in one which received benzene. Anemia with leucocytosis was observed in the animal which received phenylhydrazine. Pathologic findings were not conclusive.

3. It is suggested that in some people transformation products possessing toxic properties are formed; and that by virtue of individual hypersensitivity, these toxic properties result in arrested maturation of the myeloid elements.

The author wishes to express his gratitude for the valuable aid and advice received from Dr. Robert M. Isenberger, Dr. J. C. Rice, and Miss Esther Rodewald.

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## SELENIUM AS A CATALYST IN THE DETERMINATION OF NONPROTEIN NITROGEN IN BLOOD\*

FREDERICK REIS, M.D., AND HARRY H. POWERS, PH.D., BOSTON, MASS.

SELENIUM was first introduced as a catalyst in Kjeldahl digestions by M. F. Lauro,<sup>1</sup> and its use as such suggested to us the possibility of its being used in the determination of nonprotein nitrogen in blood filtrates, our aim being to develop a method of digestion as rapid and as convenient as the Folin-Wu method<sup>2</sup> but without the troublesome formation of a precipitate of silica, due to the action of the phosphoric acid of their digestion mixture on glass. While this precipitate may be avoided by a careful regulation of the flame, its occurrence is always possible, and when present, interferes with colorimetric readings unless removed by centrifuging.

As a result of several preliminary experiments, it was found that 45 per cent sulphuric acid containing a small amount of selenium could be substituted for the Folin-Wu digestion mixture with very little sacrifice of the length of digestion time, and with no formation of silica precipitates. Either selenium or selenious acid may be used. The best results were obtained with a solution of 70 mg. selenium in 200 c.c. of 45 per cent sulphuric acid.

The digestion is carried out in much the same way as the Folin-Wu method. In place of the charring seen in the latter method, a rust-colored precipitate of reduced selenium appears. This dissolves as the heating is continued, the digest becoming practically colorless within thirty seconds to three minutes after the appearance of the fumes of sulphur dioxide. Five minutes of heating from the first appearance of the acid fumes is sufficient for the complete digestion of most urine samples, and for all blood filtrates unless these filtrates, through improper preparation, contain traces of protein. In such filtrates in the Folin-Wu method, the protein becomes charred and sticks to the side of the digestion tube so that it is not completely digested. In the selenium sulphuric acid method this trace of protein goes into solution, but is quite difficult to digest. Usually an additional five minutes' heating completes the digestion in these cases, and if a slight brown color still persists after ten minutes' digestion, it may be disregarded as it apparently does not affect the accuracy of the determination.

Using the selenium sulphuric acid digestion method for the determination of total urinary nitrogen, analyses were done on urine and on known solutions of urea and uric acid. For the urine a value of 10.7 gm. nitrogen per liter was obtained as compared with 10.64 gm. with the Folin-Wu method. The urea solution gave a value of 3.35 mg. nitrogen per 100 c.c. as compared with the

\*From the Department of Biological Chemistry, Tufts College Medical School.  
Received for publication, January 16, 1935.

calculated value of 3.5 mg. A lithium carbonate solution of uric acid on one analysis gave a value of 3.29 mg. nitrogen per 100 c.c. and on a second analysis, 3.33 mg. as compared with a calculated value of 3.33 mg.

TABLE I

NONPROTEIN NITROGEN OF BLOOD SHOWING THE COMPARATIVE VALUES OBTAINED BY THE FOLIN WU AND SELENIUM SULPHURIC DIGESTION METHODS

SAMPLE	NONPROTEIN N, MG. PER CENT	
	FOLIN-WU	SELENIUM SULPHURIC
1	31.1	31.1
2	35.9	36.4
3	32.4	32.4
4	31.1	31.1
5	34.5	34.7
6	45.5	45.8
7	39.4	39.8
8	37.0	36.9
9	45.6	45.4
10	35.9	35.7
11	38.7	38.7
12	159.1	157.8
13	27.7	27.3
14	22.9	24.3
15	32.2	31.1

Tungstic acid filtrates, especially those from unlaked blood, usually contain varying traces of unprecipitated tungstic acid. In the Folin-Wu digestion the phosphoric acid keeps the tungstic acid in solution, but in procedures not using phosphoric acid, it separates out and results in turbidity on nesslerization. This turbidity may be prevented by the use of gum ghatti solution which was first introduced by Folin<sup>3</sup> in his ferrieyanide sugar method and later used by several different authors in methods involving nesslerization. By the use of gum ghatti we have succeeded in nesslerizing solutions that were milky with precipitated tungstic acid, although in some cases the solution would have to stand for several minutes before all of the tungstic acid would be dissolved by the alkali of the Nessler's.

#### METHOD

The method as outlined is for the determination of nonprotein nitrogen in the regular Folin-Wu filtrates. In the Folin-Svedberg micromethod<sup>4</sup> in which the blood is unlaked, the selenium sulphuric acid solution described below is diluted 1 to 5 and used in place of the regular digestion mixture, and 1 c.c. of gum ghatti solution is added prior to nesslerization. In the determination of the total nitrogen of urine the only change from the Folin method is in the substitution of the selenium sulphuric acid solution.

#### *Solutions Required.*—

1. Standard ammonium sulphate solution containing 0.1 mg. nitrogen per c.c.
2. Nessler's reagent.
3. Gum ghatti solution.

Directions for the preparation of the above solutions may be found in *Laboratory Manual of Biological Chemistry*, Folin (1934).



4. Selenium sulphuric acid solution: Dissolve with heat 70 mg. selenium in 90 c.c. of sulphuric acid, cool and dilute to a volume of 200 c.c. with water. It may also be prepared by adding 5 c.c. of 2 per cent selenious acid to 200 c.c. of 45 per cent sulphuric acid.

*Determination.*—Transfer 5 c.c. of the Folin-Wu filtrate corresponding to 0.5 c.c. of whole blood to a Pyrex test tube graduated at 50 c.c. Add 1 c.c. of the selenium sulphuric acid solution and an antinump tube. Then adjust the flame of a microburner to a height of about one inch and clamp the Pyrex tube so that the bottom is about  $\frac{3}{4}$  inch above the top of the burner. This amount of flame will insure a rapid boiling away of the water and need not be changed during the course of the digestion. When the fumes of sulphur dioxide appear, cover the mouth of the tube with a watch glass and continue the heating for five minutes, or ten minutes, if necessary. Allow to cool thoroughly and add about 25 c.c. of water.

Now place 1.5 c.c. of the standard ammonium sulphate solution containing 0.15 mg. nitrogen in a similar test tube, add 1 c.c. of the selenium sulphuric acid solution and about 25 c.c. of water. Then add 2 c.c. of gum ghatti solution to each, mix slightly by twirling the tubes, add 15 c.c. of Nessler's solution to each, dilute to the 50 c.c. mark and mix. Note: We have found it best to complete the nesslerization and mixing of each tube separately, for if a tube is left even a short time without mixing, the localized concentration of the alkaline Nessler's solution may be sufficient to start the development of turbidity.

The solutions are allowed to stand for about five minutes and then compared in the colorimeter. The calculation is the same as in the regular method:

$$\frac{20}{\text{reading of the unknown}} \times 30 \text{ equals mg. nitrogen per 100 c.c. of blood where the standard is set at 20 mm.}$$

Table I shows the comparative results obtained by the Folin-Wu and the selenium sulphuric acid methods. In Samples 13, 14, and 15, the Folin-Svedberg method and its selenium sulphuric acid modification were used. The figures given represent the average of two determinations.

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# DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

TISSUE: Preparation of Neurologic Material for Histologic Study, Kernohan, J. W. Am. J. Clin. Path. 4: 410, 1934.

## Method 1.—Fat staining method:

The methods following have been found very satisfactory.

1. Fix tissue in formol solution, freeze and section, 10 to 20 microns.
2. Wash sections in 70 per cent alcohol for one minute.
3. Stain for five minutes in fat stain, made as follows:

Sharlach R, 1 gm. (Sudan IV may also be used)

Absolute alcohol, 70 c.c.

Distilled water, 30 c.c.

(Heat this mixture just a little, and shake while heating. Then add 100 c.c. acetone and put in a 37° C. oven overnight. Always filter stain before using.)

4. Wash in 70 per cent alcohol quickly.
5. Stain in hematoxylin (Harris) for five minutes.
6. Wash in tap water.
7. Dip in (1 per cent) acid (70 per cent) alcohol about two seconds.
8. Wash in tap water ten minutes or longer.
9. Wash in lithium carbonate (saturated aqueous solution) about two seconds.
10. Wash in tap water and mount with glycerin.

## Method 2.—Perdrau impregnation method:

1. Paraffin sections run down to water in usual way.
2. Place for twenty to thirty minutes in 0.25 per cent solution of potassium permanganate.
3. Wash in water.
4. Place in solution of equal parts of 1 per cent oxalic acid and 1 per cent potassium sulphite, or 5 per cent oxalic acid, until color is gone.
5. Wash for a half to one hour in tap water until all acid is washed out (limit the length of time in case the sections come loose).
6. Place sections in a 20 per cent solution of silver nitrate for half an hour.
7. Wash quickly in double-distilled water.
8. Place sections in the following solution:  
Silver nitrate (20 per cent) solution, 5 c.c.  
Sodium hydroxide, 6 drops.

(Add ammonium hydrate drop by drop until the precipitate is almost destroyed. Add distilled water up to 50 c.c. and filter. Leave sections in this solution for half an hour.)

9. Wash in double-distilled water.
10. Fix in 10 per cent formol solution (neutral).
11. Wash in double-distilled water.
12. Tone in gold chloride solution 1:500.
13. Fix in sodium hyposulphite.
14. Wash in tap water, dehydrate, clear, and mount.

## Method 3.—Toluidine blue, or thionin, staining method:

1. Run sections down to water.
2. Place sections in toluidine blue or thionin, stain for fifteen to twenty minutes (heat stain before using for thirty minutes): 1:500 solution = 1 gm. toluidine blue or thionin to 500 c.c. water.

3. Wash in two changes of 95 per cent alcohol (color comes out rapidly).
4. Place in absolute alcohol and watch under microscope until differentiation is complete.

5. Clear in xylol, thoroughly, and mount with Canada balsam.

*Method 4.*—Modified Bielschowsky impregnation method:

1. Fix tissue in solution of formol, 10 per cent.
2. Embed section in paraffin and cut at 10 microns.
3. Deparaffinize.
4. Wash three times in double-distilled water.
5. Place in 20 per cent solution of silver nitrate for one hour.
6. Wash quickly (twice) in double-distilled water.
7. Transfer sections into ammoniated solution of silver nitrate for five minutes: To 40 c.c. of 20 per cent silver nitrate solution add ammonium hydrate drop by drop until precipitate is dissolved. An excess of ammonium hydrate is noxious. Make a fresh solution each time it is to be used. Filter before using.

8. Wash quickly in double-distilled water.

9. Transfer sections into 10 per cent solution of formol (neutral) for one minute.

10. Wash, tone in gold chloride (brown) solution (a), fix in sodium hyposulphite solution (b) one or two minutes, clear (c), and mount (d):

(a) Gold chloride, 1 gm.

Water, 500 c.c. (can be kept as stock solution).

(b) Sodium hyposulphite, 5 gm.

Water, 100 c.c.

(c) 95 per cent alcohol.

Acetone.

Carbo-xylol.

Xylol.

(d) With Canada balsam.

*Method 5.*—Mallory-Heidenhain's staining method:

1. As a fixative, use formol or Zenker's solution, or Weigert's mordants. Cut paraffin sections at 5 microns.

2. Stain forty minutes in:

Azo-carmin (Grubler's), 1 gm.

Water, 100 c.c.

(Heat, cool, and filter at room temperature and add 1 c.c. of glacial acetic acid before using.)

3. Wash in water.

4. Differentiate in aniline alcohol (watch under microscope) until nuclei are red and cytoplasm is pale pink. This step requires from three to ten minutes depending on thickness of sections.

5. Remove aniline alcohol with acid alcohol (1 per cent) a half to one minute.

6. Wash quickly in water.

7. Place in 5 per cent phosphotungstic acid for three hours.

8. Wash quickly in water.

9. Stain one-fourth to one-half hour in Mallory's aniline blue made as follows:

Aniline blue, 0.5 gm.

Orange G, 2 gm.

Water, 100 c.c.

Acetic acid, 8 c.c.

(Boil, cool, filter, and thin with equal parts of water before using.)

10. Wash quickly in water.

11. Differentiate in absolute alcohol.

12. Clear in carbo-xylol and xylol.

13. Mount with Canada balsam.

*Method 6.*—Mallory's phosphotungstic acid-hematoxylin staining method:

1. Fix in Zenker's solution for twenty-four hours.
  2. Place in running water for twenty-four hours.
  3. Embed in paraffin.
  4. Section and deparaffinize.
  5. Place in potassium permanganate for from five to twenty minutes (make a fresh 0.25 per cent aqueous solution each time).
  6. Wash well in water.
  7. Place in oxalic acid (5 per cent aqueous solution) for from five to ten minutes.
  8. Wash thoroughly in several changes of water.
  9. Stain with Mallory's phosphotungstic acid-hematoxylin stain three to twelve hours.
- This stain is made as follows:

Ammonia hematin, 0.1 gm.

Water, 100 c.c.

Phosphotungstic acid crystals (Merck), 2 gm.

(Dissolve the ammonia hematin in a little water, with the aid of heat, and add it when cool to the rest of the solution; no preservative is required. If the solution stains weakly at first, it may be ripened by the addition of 5 c.c. of a 0.25 per cent aqueous solution of potassium permanganate, or it may be allowed to stand for a few weeks until it ripens spontaneously. Hematoxylin may be used instead of ammonia hematin, but requires 10 c.c. of the permanganate solution to ripen it.)

10. Place sections in 95 per cent alcohol, then in absolute alcohol, and clear in xylol.
11. Mount with Canada balsam.

*Method 7.*—Weigert's mordant I:

Potassium bichromate, 5 gm

Fluorochrome, 2 gm.

Water, 100 c.c.

(Fix tissue in mordant I for from four to five days, for small and thin sections, two days.)

*Method 8.*—Weigert's mordant II:

Acetate of copper, 5 gm.

Fluorochrome, 2.5 gm.

Acetic acid (36 per cent), 5 c.c.

Water, 100 c.c.

Formol, 10 c.c.

(Fix tissue in mordant II for from twenty-four to forty-eight hours.)

*Method 9.*—Weigert's myelin-sheath staining method:

1. Wash tissue, which has been fixed in 10 per cent solution of formol, in water for short time.
2. Fix in primary mordant four to five days (for small and thin sections, two days).
3. Wash in water one hour.
4. Fix in secondary mordant twenty-four to forty-eight hours.
5. Wash well in water one to two hours.
6. Embed sections and deparaffinize.
7. Stain two hours in a solution made as follows:

10 c.c. of a 10 per cent solution of hematoxylin dissolved in absolute alcohol.  
Saturated solution of lithium carbonate, few drops.  
Distilled water, 90 c.c.

(Make this solution fresh each time.)

8. Wash in tap water.

9. To differentiate, place in a solution of:

Borax, 2 gm.

Potassium ferrieyanide, 2.5 gm.

Water, 100 c.c.

10. Wash thoroughly in running water.
11. Dehydrate in 80 per cent and in 96 per cent alcohol.
12. Clear in acetone, carbo-xytol, and xytol.
13. Mount with Canada balsam.

(A stock solution of 10 per cent solution of hematoxylin in absolute alcohol should be kept on hand. At least ten days' exposure to sunlight is required to ripen this solution. Combine it with a few drops of saturated aqueous solution of lithium carbonate at the time of using.)

*Method 10.*—Cajal's gold chloride and sublimate method:

1. Place small portions of fresh tissue in the following solution for two to ten days (the tissue may have previously been fixed in formal solution a very short time):

Formol, neutral, 15 c.c.

Ammonium bromide, 1.5 to 2 gm.

Distilled water, 85 c.c.

(Tissue should not remain more than ten days in this solution, seven days is best.)

2. Cut sections on freezing microtome (20 to 25 microns) and place in 6 per cent neutral formol solution.

3. Wash a few moments in distilled water.

4. Place in following solution for six to ten hours and keep in the dark:

Gold chloride (1 per cent), 10 c.c.

Mercuric chloride, 0.5 gm. (10 c.c. of a 5 per cent solution).

Distilled water, 60 c.c.

(Dissolve the mercuric chloride by heat in nearly all water called for in the formula, filter, and add to the gold chloride. The best temperature is 18 to 20° C. At this temperature, four to six hours is the best. If temperature is 14 to 17° C., the time should be much longer. If temperature is higher, the time should only be two to three hours.)

5. After four or more hours, when sections are intense purple, fix for from six to ten minutes in the following solution:

Sodium hyposulphite, 5 c.c.

Alcohol (95 per cent), 30 c.c.

Distilled water, 70 c.c.

Saturated solution bisulphite of soda, 5 c.c.

6. Wash in 50 per cent alcohol.

7. Place on slide, blot carefully, then dehydrate on slide with 70 per cent, and 95 per cent, and absolute alcohol; then clear in xytol.

8. Mount in Canada balsam.

(Blot carefully after each of above steps. Oil of origanum may be used in place of absolute alcohol.)

*Method 11.*—Hortega silver carbonate impregnation method for oligodendroglia and microglia:

1. Fix tissue in formal-ammonium bromide solution (same as for Cajal's stain) and cut frozen sections.

2. Wash in two dishes of distilled water, the first containing 10 drops of ammonia.

3. Stain in undiluted silver carbonate for from one minute to one and a half hours.

Del Rio Hortega's undiluted ammoniacal silver carbonate is prepared as follows:

Solution of silver nitrate (Merck) 10 per cent, 5 c.c.

Solution of sodium carbonate (pure) 5 per cent, 20 c.c.

Ammonium hydroxide, sufficient to dissolve precipitate.

(The ammonium hydroxide should be added drop by drop until the precipitate is just dissolved, stirring the solution all the while.) Finally, filter the solution and place in a dark bottle, where it will keep for long periods.

4. Wash rapidly in 60 per cent alcohol (made by using absolute alcohol). The sections should be carried through with a small angulated glass rod so as to allow all of it to be washed equally without wasting time. If the section is wrinkled or folded, the alcohol will produce a patchy result.

5. Reduce by passing sections directly in 1 per cent formol solution.

6. Wash in distilled water.

7. Tone by placing sections in a gold chloride bath ten to fifteen minutes until they become purple gray.

Gold chloride (yellow), 1 gm.

Distilled water, 500 c.c.

8. Fix in a 5 per cent solution of sodium hyposulphite for half a minute or more until sections are flexible.

9. Wash in water.

10. Dehydrate in dishes of graded alcohol and clear in carbo-xylol and xylol.

11. Mount with Canada balsam.

**LIVER FUNCTION, In Pregnancy, The Bilirubin Excretion Test In, Sullivan, C. F., Tew, W. P., and Watson, E. M. Brit. J. Obst. & Gynec. 41: 347, 1934.**

The investigation of liver function by means of the bilirubin excretion test of von Bergmann in pregnancy and its complications, as described in this paper, permits the following inferences:

1. During the first half of normal pregnancy the liver function, as determined by the bilirubin excretion test, is unimpaired, but during the second half of normal pregnancy, evidence of disturbed function can be demonstrated in at least 30 per cent of cases.

2. The cause of the impaired excretory power of the liver during the later stages of normal pregnancy is undetermined, but the fact of its existence renders the interpretation of hepatic efficiency tests in abnormal cases difficult.

3. Toxic patients, with signs of renal insufficiency, tend to show less retention of injected bilirubin than those with normal kidney functions. Thus, to some extent at least, it is possible to differentiate the toxemias of pregnancy into nephritic and hepatic types. So far as the bilirubin excretion test is concerned, there is not sufficient difference between the findings in toxic patients and the results obtained in normal women during the later months of pregnancy to be of practical significance. In certain instances, however, especially during the early stages of pregnancy or in cases of extreme toxemia, the test may supply information of clinical importance.

4. That the impairment of liver function which occurs during pregnancy, both normal and abnormal, is of a temporary nature is indicated by the tendency for the results of the bilirubin excretion test to return to normal following termination of the pregnancy.

5. Owing to its limited usefulness and the technical difficulties involved, the bilirubin excretion test is unlikely to become popular as a routine procedure in the practice of obstetrics.

The test procedure is as follows:

An amount of bilirubin equivalent to 1 mg. per kilogram of body weight is dissolved in 15 c.c. of 0.1 M. solution of sodium carbonate, previously boiled and cooled to about 80° C. After the pigment has dissolved completely the solution, having further cooled nearly to body temperature, is transferred to a 20 c.c. syringe and injected slowly into a vein at the elbow. Untoward effects of any kind have not been observed to follow such injection. Blood samples are obtained immediately before the injection and four minutes, thirty minutes, two hours, and four hours afterward. These samples, which are received in centrifuge tubes containing potassium oxalate, are centrifuged at once and kept in a refrigerator until the final collection has been made.

The concentration of bilirubin in each sample of blood plasma is estimated by the method of Ernst and Foster which procedure consists essentially of the simultaneous precipitation of the plasma proteins and the extraction of the bilirubin with acetone. For this

purpose 2 c.c. of acetone are added to 1 c.c. of plasma in a centrifuge tube. After shaking the plasma and acetone mixture, the precipitate is removed by centrifugalization and the supernatant liquid which contains the pigment is filtered directly into a microcolorimeter cup through a No. 40 Watman filter paper and compared with a standard composed of 1 in 6,000 potassium dichromate. It was found that clearer filtrates could be obtained if the centrifuge tubes, following the removal of the protein, were stoppered and chilled by immersion in a water-bath containing lumps of ice for five minutes before filtration. The acetone solutions must be protected from the light previous to making the color comparisons.

It is true that this method of estimation includes not only the bilirubin but other pigments such as carotin and lipochromes should these be present in the plasma, but in the cases reported in this paper, any effect produced by such substances was minimized by carrying out the tests with the patients in the fasting stato. Moreover, assuming any error due to the presence of these nonbiliary pigments to be constant throughout the period of the test, their influence is removed completely by subtracting the result obtained in the preliminary control sample from the values found in the subsequent ones. Hence the bilirubin content of the specimen secured four minutes after the injection of the bilirubin minus that of the control sample is regarded as representing the maximum increment caused by the added pigment and is consequently recorded as 100 per cent. With this value as a basis, the findings for the other samples are expressed accordingly. While the results so obtained represent the relative rather than the absolute bilirubin concentrations, they do provide a representation of the completeness of elimination of the injected bilirubin in a specified time.

**BLOOD SEDIMENTATION, Meteorologic Effects On, Hoverson, E. T., and Petersen, W. F.**  
Am. J. M. Sc. 188: 455, 1934.

There occur wide daily variations in the erythrocyte sedimentation rate, and at times the daily variation is as much as 100 per cent.

In general paresis, the rate of settling is slower than in "normal" individuals.

There is a correlation between the daily variations of the sedimentation time and the meteorologic changes.

It is believed that these meteorologic changes account for daily variations in the rate of settling.

**PERIARTERITIS NODOSA, Associated With Rheumatic Heart Disease, Friedberg, C. K., and Gross, L.** Arch. Int. Med. 54: 172, 1934.

Four cases that came to autopsy are presented, in which widespread periarteritis nodosa was associated with rheumatic fever and rheumatic heart disease; the latter was confirmed by the presence of Aschoff bodies in the myocardium.

These four were discovered in a series of eight cases of periarteritis nodosa which came to autopsy in the course of two years. Prior to this period there were five additional cases which came to autopsy. Two of the five patients had a rheumatic history and evidence of rheumatic valvular disease. Verrucous endocarditis was disclosed in both cases at postmortem examination.

Criteria for the diagnosis of rheumatic infection and of periarteritis nodosa are discussed. On the basis of these criteria, none of the cases of periarteritis reported in the literature presented adequate evidence of rheumatic heart disease. Conversely, none of the vascular lesions described in rheumatic fever could be truly called periarteritis nodosa.

Because of the frequency of the association of these diseases in our cases and the simultaneous occurrence of the symptoms of each, we believe it probable that rheumatic fever is a common cause of the vascular lesions termed periarteritis nodosa.

In two of the cases an attack of scarlet fever occurred eight weeks before the symptoms of the other ailments became manifest. This point is briefly discussed.

In another case there was clinical and pathologic evidence of malignant sclerosis. This is mentioned in connection with Fahr's belief that rheumatic fever is one of the causes of malignant sclerosis.

In two of the cases the abdominal symptoms, so common in periarthritis nodosa, dominated the clinical picture sufficiently to lead to an exploratory operation. The authors suggest that when acute abdominal symptoms are present in a patient suffering from rheumatic fever, complicating periarthritis nodosa should be considered. This complication is offered as an organic basis for some of the instances of so-called abdominal rheumatism.

**HOOKWORM, Comparison of Efficiency of Stoll Egg-Counting Technic With Simple Smear Method in Diagnosis of, Keller, A. E. Am. J. Hyg. 20: 307, 1934.**

In order to compare the efficiency of the Stoll egg-counting technic with the simple smear method in the diagnosis of hookworm, 2,412 specimens of feces were examined according to the following plan: Each specimen was examined one time by the dilution egg-counting technic and the simple smear method. The specimens which were positive by egg count but negative by smear or positive by smear and negative by egg count were reexamined by the method which failed to reveal hookworm eggs on the first examination.

On one examination 42.2 per cent of the specimens were positive by the egg-counting technic and 35.1 per cent were positive by the smear method. Forty-five additional positive specimens were found by the egg-counting examination which increased the total positive by egg count to 44 per cent. One hundred four additional positive specimens were found by the smear examination, increasing the total positives to 39.4 per cent.

In this series of examinations 7.1 per cent more specimens were found positive by one egg count than by one smear examination and 4.6 per cent after reexamination of the negatives which were not diagnosed by the first smear examination.

The greatest discrepancy between the two methods occurred in the lowest intensity groups where from 57.8 to 68 per cent of the positive specimens by egg count were positive by the smear method. When the intensity of infestation reached 2,000 eggs per gram of feces 95.1 per cent of all the specimens found positive by egg count were positive by smear. All of the specimens in the intensity group containing 5,000 eggs or more per gram of feces were positive by smear.

It was found for this series that the lowest level of intensity of infestation at which the smear, for practical purposes, would be of value in the diagnosis of hookworm infestation would be 1,200 eggs per gram of feces rather than 500 eggs per gram as has been determined by other investigators.

The positive specimens missed by two egg counts were as a rule light infestations (50 worms or less) but an occasional high count would be missed by this technic.

A correlation of the relative number of eggs per smear with the intensity of infestation as found by the egg-counting technic was made of 931 specimens. In the smear group containing from one to five hookworm eggs per slide there was an average count of 1,690 eggs per gram (approximately 65 worms). In the smear group containing from 6 to 25 eggs per slide the average intensity of infestation was 4,190 eggs per gram (approximately 160 worms) and in the group containing from 26 to 40 eggs per slide by the smear method the average count was 12,325 eggs per gram (approximately 492 worms). When there were 41 or more eggs per slide by smear examination the average egg count was 23,100 eggs per gram (approximately 924 worms).

These results suggest that it may be possible to correlate the number of hookworm eggs found per smear with the intensity of infestation as found by the dilution egg count examination.

These data show that the dilution egg-counting technic is more accurate than the smear method for this series of examinations and that the results of investigations which have been conducted in which the Stoll egg-counting technic has been employed are dependable when compared with the results obtained by the examination of specimens by the smear method.

**PNEUMOCONIOSIS, Pathology of: Review, T.H.B. Am. J. M. Sc. 188: 418, 1934.**

From the data assembled and discussed in this rather comprehensive review the following conclusions are drawn:



There is no conclusive evidence to show that silica has a specific action on the tissues.

Certain of the silicates and perhaps other substances may produce similar if not identical effects.

Infection, and especially tuberculous infection, is a more serious factor in the development of the disabling pneumoconioses than dust per se.

Dust modifies pulmonary tuberculosis in a twofold manner; it increases the fibrous response, thus promoting chronicity, and at the same time it increases the tendency of the infection to spread.

The so-called "silicotic nodule" has probably never been reproduced experimentally except by the synchronous inoculation of tubercle bacilli with dust.

The "silicotic nodule" is probably always a tuberculous lesion, modified by dust.

Chemical assay of suspected tissues is open to the criticism that it does not reveal the proportion of silica to silicates.

There is probably no such thing as acute silicosis.

Evidence regarding the action of coal dust on the tissues and its influence on tuberculous infection is conflicting.

**TRICHINOSIS, Anomalous and Nonspecific Reactions With *T. Spiralis* Antigen in Relation to Other Disease Conditions, Bachman, G. W., Molina, R. R., and Gonzalez, J. O. Am. J. Hyg. 20: 415, 1934.**

The fact that human trichiniasis has not been found in Porto Rica enabled the authors to study the specificity of the precipitation test in relation to other disease conditions existing in the Island.

Anomalous and nonspecific precipitation reactions were studied in serums from 857 apparently trichinella-free individuals living in Porto Rica. These individuals were classified into 6 groups: A, B, C, D, E and F, with relation to the various disease conditions affecting them at the time the test was made.

From these 6 groups 857 serums were tested, of which 35 (or 4.8 per cent) gave an anomalous type of reaction, 72 (or 8.4 per cent) gave nonspecific precipitation reactions, and 750 (or 87.5 per cent) gave negative reactions.

Group A: In 98 individuals who gave a positive Wassermann and Kahn reaction, 2.0 per cent gave anomalous types of reaction, and 8.1 per cent gave nonspecific precipitation reactions. In 573 individuals, negative for Wassermann and Kahn, 0.34 per cent gave anomalous reactions, 4.7 per cent gave nonspecific reactions, and 94.9 per cent were negative.

Group B: In 39 individuals of 81 malaria cases (positive laboratory diagnosis), all of whom had received quinine, 7.69 per cent gave anomalous reactions, 12.9 per cent, nonspecific reactions, and 79.48 per cent, negative reactions. Of the remaining 42 individuals who did not take quinine, 9.52 per cent gave anomalous reactions, 26.19 per cent gave nonspecific reactions and 64.28 per cent gave negative reactions. The results obtained in this group seem to indicate that a positive or negative history of quininization previous to the precipitation tests had no effect on the number of anomalous reactions.

Group C: In 42 individuals clinically diagnosed as malarial cases, 2.38 per cent gave anomalous reactions, 2.38 per cent, nonspecific, and 95.2 per cent, negative. In this group the lowest percentage (2.38 per cent) of nonspecific reactions was found for all the groups.

Group D: In serums from 169 individuals harboring one or more intestinal nematodes, only one case that harbored *Necator americanus* gave an anomalous reaction and one nonspecific reaction. None of the cases harboring *Ascaris* or *Trichuris* gave either anomalous or nonspecific precipitation reaction.

Group E: In the serums from 154 hospitalized cases suffering from one or more disease conditions, there were no anomalous reactions. Fourteen (9.09 per cent) gave nonspecific reactions, and 140 (90.9 per cent), negative.

Group F: In the serums from 7 individuals who showed nitrogen retention, increased cholesterol and chlorides in their blood, two anomalous reactions occurred. In this small group 71.4 per cent gave nonspecific precipitation reactions, the highest observed in all of the 6 groups.

Of the 857 serums studied from the above groups, the titers varied from 1:100 to 1:3,000. In the precipitation test of the various groups, 18.06 per cent gave a titer of 1:100; 19.4 per cent, 1:200; 41.6 per cent, 1:500; 11.9 per cent, 1:1,000; 4.0 per cent, 1:1,500; 2.8 per cent, 1:2,000; 2.08 per cent, 1:3,000.

We may deduce from these investigations that the precipitation test for the diagnosis of human trichiniasis does possess a fairly high specificity in relation to other disease conditions. It is the experience of the authors that the presence of the anomalous reactions can be easily differentiated from the true, positive precipitation rings. According to the results of the authors, nonspecific precipitation reactions occur in low dilutions at the interphase of the serum and test antigen, and give rings similar to a true precipitation ring in Wassermann and Kahn reactions and parasite infestations (Group E) as well as in conditions where there is nitrogen retention and increased cholesterol and chlorides in the blood.

A titer of 2,500 and above in terms of dry weight of powder can, according to the experience of the authors, be termed specific for trichiniasis in 90 per cent of cases.

### Erratum

In the article by William P. Belk in the July issue of THE JOURNAL OF LABORATORY AND CLINICAL MEDICINE, page 1035, the author's name and title should read:

William P. Belk, M.D., Ardmore, Pa.

Instructor in Clinical Biochemistry, Graduate School of Medicine, University of Pennsylvania.

The following bibliography is added:

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# The Journal of Laboratory and Clinical Medicine

VOL. 20

AUGUST, 1935

No. 11

Editor: WARREN T. VAUGHAN, M.D.

Richmond, Va.

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## EDITORIAL

### Vaccination Against Acute Anterior Poliomyelitis

THAT monkeys can be successfully vaccinated against acute anterior poliomyelitis by subcutaneous and intracutaneous injections of the virus has been amply demonstrated,<sup>1</sup> but the method has been considered too risky for the immunization of human beings.

Kolmer and Rule,<sup>2</sup> however, have found that monkeys can be safely and very effectively vaccinated with virus slightly attenuated by sodium ricinoleate. Subcutaneous injections of amounts as high as 0.05 to 1 c.c. per kg. of a vaccine of 4 per cent suspensions of monkey spinal cord virus in 1 per cent solutions of this agent for as many as ten consecutive injections, have produced no evidence of infection while immunizing the animals to intracerebral injections of ten or more infective doses of highly virulent virus. Furthermore, the immunity has been found to endure for at least three years.

Following the experiments these investigators took three subcutaneous injections of the vaccine with no ill effects and subsequently administered it to a group of twenty-five children varying in age from eight months to fifteen years by subcutaneous injection at weekly intervals for 2 to 3 doses.<sup>3</sup> Only slight reactions occurred at the sites of inoculation with no ill effects and with the production of antiviral antibody in the blood of about 85 per cent believed to be sufficient for affording protection against the disease.

Since then over 5,000 children have been immunized in different parts of the country<sup>4</sup> but especially in North Carolina, Virginia, Tennessee and neighboring states where an epidemic began in June. Only slight local reactions have occurred at the sites of injection although a very careful aseptic technique must be observed in its administration because of the low bacteriostatic and bactericidal effects of sodium ricinoleate. An occasional severe local reaction has been found due to accidental bacterial contamination of the vaccine, although 1:60,000 phenyl mercuric nitrate is now being added which has been found sufficient for bacteriostatic effects and without appreciable reduction in the immunizing properties of the vaccine.<sup>4</sup>

It would appear therefore that this vaccine of living attenuated virus has been proved safe for the immunization of human beings. Furthermore, the brain and spinal cord of every monkey removed at autopsy are being tested for the possible presence of the virus of lymphocytic choriomeningitis of Armstrong and Lillie<sup>5</sup> by intracerebral injection into mice and guinea pigs before the cord is used in the preparation of the vaccine, but so far tests with a large number of monkeys have not shown the presence of this virus.<sup>4</sup> It is believed by Kolmer and his associates that the safety of the vaccine may depend upon the possibility and probability that the remote monkey passage virus employed in its preparation has lost infectivity for human beings. Furthermore, the subcutaneous route of administration, a small first dose, and the rapidity of antibody production are believed to be additional factors of safety. While attenuation of the virus by sodium ricinoleate may be an additional factor of safety, yet the degree of attenuation is so slight as to be a factor of much lesser importance.

Whether or not the effectiveness of the vaccine in the prevention of infantile paralysis will be as definitely shown as has its safety remains to be determined. But the fact that it has proved highly effective in the protection of monkeys and that the antibody it has engendered has neutralized human virus from the California epidemic<sup>3</sup> appears to indicate that it will prove highly effective. While Kolmer and his associates have found antiviral antibody in the blood of some children as early as four days after the first injection,<sup>3</sup> it may be that it will fail to abort the disease when given late in the incubation period.

Vaccines of virus killed by heat and various chemical agents have proved much less effective in the immunization of monkeys. Park and Brodie of New York, however, have found that large doses of a 20 per cent suspension of monkey spinal cord virus believed to be "killed" with 0.1 per cent formalin were effective in the immunization of monkeys and that two doses by sub-

cutaneous and intraocular injection produced antibody lasting for about five months in the blood of about 85 per cent of children. This vaccine has been used to some extent in the California epidemic and is also being tried in the North Carolina epidemic at the present time. As stated by Andrewes,<sup>6</sup> however, it would appear definitely established that vaccines of dead viruses are enormously weaker than living and attenuated viruses because the former contain but small amounts of antigenic virus protein, while the virus in the latter after injection can multiply, perhaps, a million fold in the body and thereby have a far better chance of producing resistance.

If, therefore, the Kolmer vaccine is proved completely safe, as appears to be the case, it may prove to be the more effective and capable of engendering a greater degree of immunity of longer duration. At any rate both vaccines are now being given a thorough trial in the North Carolina epidemic and the results will be awaited with a great deal of interest. And, while the attack rate of the disease is normally low, yet this is so much increased during epidemics that it is greatly hoped that one or both of these vaccines will prove effective in protecting children over the age of greatest susceptibility until the natural immunity occurring in about 80 per cent of adults has been acquired.

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—W. T. V.

# *The Journal of Laboratory and Clinical Medicine*

VOL. 20

SEPTEMBER, 1935

No. 12

## CLINICAL AND EXPERIMENTAL

### SURGICAL MAGGOTS\*

#### A STUDY OF THEIR FUNCTIONS IN WOUND HEALING

FREDERICK C. MESSER, B.S., AND ROBERT H. MCCLELLAN, M.D., PITTSBURGH, PA.

#### INTRODUCTION

THE value of blowfly larvae in the treatment of chronic osteomyelitis and of other infected wounds is becoming more widely appreciated, and surgeons are using them with increasingly greater confidence.<sup>12</sup> Little has been learned, however, as to the specific ways in which they influence wound healing, or the means by which they are able to exert such influence. Those who have had opportunity of observing larval therapy agree in general that when placed in infected wounds, maggots decrease the bacterial population, remove necrotic tissue, and stimulate healing. There is unfortunately little exact data to prove causal connection between the presence of maggots and any of the above effects. Some objective evidence exists to support the first, but the latter two are founded on clinical observation. In the last analysis, they must remain a matter of opinion until controlled experimental data support them.

Since observations on the healing of clinical, and even of experimental lesions, are difficult to control, it would seem more promising to study certain phases of the physiology of blowfly maggots, in order to demonstrate in what manner, and to what degree they could produce their alleged effects. We have chosen this latter mode of attack in the work reported in this paper.

\*From the John C. Oliver Memorial Research Foundation, St. Margaret Memorial Hospital.  
Received for publication, February 25, 1935.  
Read at a meeting of the American Association for the Advancement of Science at Pittsburgh, December 31, 1934.

THE SOURCE OF pH CHANGES IN OSTEOMYELITIS WOUNDS TREATED WITH  
BLOWFLY MAGGOTS

Baer<sup>1</sup> made the striking observation that wounds treated with maggots became sufficiently alkaline to turn red litmus blue. Litmus is completely transformed to its blue alkaline modification at about pH 8.3,<sup>2</sup> but has a bluish tinge at lower alkalinities. Consequently, this indicator gives only a vague idea of the actual hydrogen ion concentration. In order to study more precisely the reaction of wounds healing in the presence of maggots, the hydrogen ion concentration of a number of such wounds, and of wounds of other types treated only with neutral wet dressings, was followed electrometrically over extended periods of time.

Our earlier measurements were made upon exudate from the wound cavity aspirated into an oiled tuberculin syringe without a needle, and transferred directly to the quinhydrone electrode apparatus of Geselle.<sup>4</sup> Later measurements were made by means of contact electrodes projecting into the wound, permitting readings when the quantity of exudate was too slight to be aspirated. Both systems were checked against Clark and Lubb's buffers at three different reactions, and found to agree within 0.2 pH.

Fig. 2 shows fluctuations in hydrogen ion concentration encountered in five operative wounds in chronic osteomyelitis treated with maggots, and in two open wounds of other natures dressed only with wet packs of sterile physiologic salt solution. From the osteomyelitis curves two observations may be made; first, that in all but one case the hydrogen ion concentration fluctuated widely; and second, that in every case, it at some time reached a reaction more alkaline than pH 7.4. In the two cases not treated with maggots, the fluctuations were not so marked, nor did they ever extend above pH 7.4. We have followed the hydrogen ion concentration in eight cases of chronic osteomyelitis and have found that in only two (neither of which could be followed to complete healing) was the maximum pH lower than 7.4. The highest maximum, that of Case 2, was 7.86, while the lowest (in a case followed only through the initial stages of healing) was 6.96.

We further noted in all cases that the fluctuations in pH followed roughly the clinical condition of the wound; progressive healthy healing being accompanied by a consistent increase in alkalinity, while failure to progress toward healing, or an increase of exudate, was followed by marked fluctuation toward acidity.

The increase in alkalinity beyond the reaction of blood plasma (pH 7.4) might arise from an abnormal reaction of the body tissues to the presence of maggots, or might be furnished by the maggots themselves. Specialized cells in the body are capable of secreting fluids that are relatively strongly acid (gastric juice), or strongly alkaline (pancreatic juice). One would not expect, however, such abnormal secretions from cells and organs not specialized for the purpose. On the other hand, it is known that maggots produce ammonia. Hobson<sup>6</sup> demonstrated its presence in an extract of midguts of blowfly larvae in the absence of ammonia-forming bacteria by means of Nessler's solution. To determine if sufficient ammonia could be formed by maggots to render wounds

alkaline to the degree which we have observed, the ammonia production of sterile blowfly larvae was measured quantitatively.

Air, aspirated through the apparatus illustrated in Fig. 1, passed first through sulphuric acid 1:6, to remove atmospheric ammonia (*A*), then in turn through jar *B* which contained the maggot culture bottle, and over glass wool saturated with 50 per cent sodium hydroxide in *C*. This measure was necessary to prevent excess moisture from condensing in the vertical connections leading out of the incubator, thus trapping ammonia. The air was then rehumidified by bubbling through weak sodium hydroxide solution in *D*, and passed in succession through two absorption tubes, *E* and *F*, each containing 10.0 c.c. of 0.1 N hydrochloric acid.

Larvae of *Lucilia sericata* were grown on sterile homogeneous nutrient slants consisting of casein and agar, together with yeast autolysate to furnish the neces-

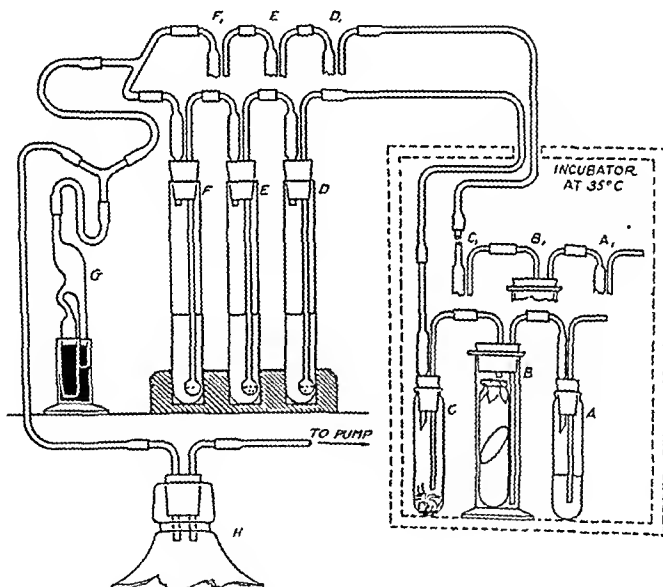


Fig. 1.—Diagram of apparatus used to measure ammonia production of blowfly larvae. (For explanation, see text.)

sary dietary factors required by for growth.\* These were contained in 50 ml. centrifuge bottles with constricted necks, the mouths of which were covered with muslin stretched tightly and tied. *Lucilia* eggs, sterilized by immersion for fifteen minutes in a solution of mercuric chloride, 1:1,000, acidified with hydrochloric acid and washed with sterile water, were seeded on one of these slants, the muslin closure was replaced, and the bottle placed in cylinder *B* in the absorption train. By means of a water pump, air was aspirated through this train continuously for a period of three to six days, during which time the eggs hatched and the maggots developed more or less toward the pre-pupal stage. To equalize fluctuations in vacuum due to variations in water pressure at dif-

\*Hobson\* has shown that blowfly larvae require for growth a food accessory contained in autolyzed yeast. We have found that the liberal addition to synthetic media of a commercial preparation of yeast autolysate, sterilized by filtration through a Seltz filter, gives excellent growth.



ferent times of day and night, it was found necessary to include in the line a mercury valve, *G*, and a five-gallon carboy, *H*, which acted as an air reservoir. A similar train, containing a culture bottle, but without eggs, was placed in parallel as a control ( $A_1, \dots G_1$ ).

At the end of the chosen period both the bottle containing the maggots and the control bottle were removed from the train and acidified with a few cubic centimeters of 0.1 N hydrochloric acid. The maggots were removed by means of forceps, washed, dried on filter paper, counted, and weighed as a group. The washings were added to the culture bottle, which was heated on a water-bath to melt the agar and diluted to 250 c.c. The control bottle was similarly treated. The standard acid in the absorption tubes was titrated, and ammonia nitrogen determined on 25 c.c. aliquots of the solution of the maggot medium and of the control medium, using Sachsse's method<sup>12</sup> of vacuum distillation in the presence of an excess of magnesia.

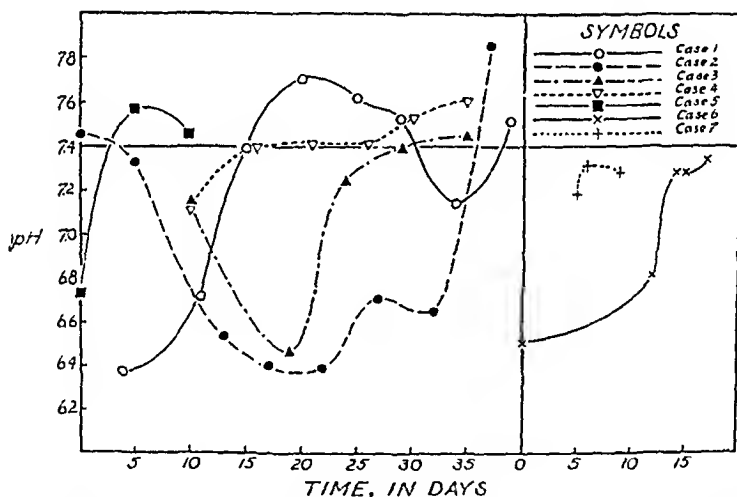


Fig. 2.—Variations in the pH of wound exudates. Cases 1 to 5, chronic osteomyelitis treated with blowfly maggots. Case 6, appendiceal abscess. Case 7, compound fracture of the tibia. Wet dressings of physiologic salt solution only were used in Cases 6 and 7.

Table I shows briefly the result of three experiments of this kind. It will be seen that under sterile conditions, each maggot is capable of furnishing at least 0.1 mg. of ammonia nitrogen per day to its surroundings, and more than this toward the end of its larval existence. The usual wound dressing in osteomyelitis contains at least 100 to 200 maggots, furnishing 10 to 20 mg. of ammonia nitrogen daily. One cubic centimeter of 0.1 N ammonium hydroxide solution, containing 1.4 mg. ammonia nitrogen, is capable of changing the reaction of 2 c.c. of blood serum over approximately one pH unit. Consequently, the amount of ammonia furnished by maggots in the usual dressing would be sufficient to neutralize much of the acid exudate characteristic of inflammation and infection, and, when the latter had to some degree subsided, to account for the excess alkalinity we have observed. It should be pointed out that maggots in the presence of bacteria produce greater quantities of ammonia than when sterile.<sup>8</sup>

The slight excess of alkalinity observed would exert a definite inhibition of bacterial growth in the wound. Since the greater part of the healing takes place while the reaction of the wound exudate is below pH 7.4, this alkalinity cannot be a great factor in early healing, but undoubtedly plays an important part in maintaining bacteriostasis, once the healing has progressed beyond a certain point. To what degree the partial neutralization of the acid inflammatory secretion aids early healing cannot be judged until we know more about conditions influencing the healing process, but it would seem likely to be beneficial to some degree.

TABLE I  
AMMONIA PRODUCTION BY BLOWFLY LARVAE

EXPER. NO.	DURATION DAYS	LIVING MAGGOTS RECOVERED	TOTAL WT. OF MAGGOTS GM.	AV. WT. OF ONE MAGGOT GM.	NH <sub>4</sub> -N IN ABSORPTION TUBES MG.	NH <sub>3</sub> -N FROM MEDIUM MG.	TOTAL NH <sub>3</sub> -N PROD. MG.	NH <sub>3</sub> -N PER MAGGOT PER DAY MG.
1	4	27	0.5763	0.0231	17.2	Not determined	17.2	0.16
2	6	55	1.8211	0.0331	27.4	20.9	48.7	0.12
3*	3	19	0.9546	0.0328	5.6	30.2	35.8	0.63

\*In Experiment 3, partly grown maggots instead of eggs were introduced into the culture bottle. The fact that they were more mature than those used in Experiments 1 and 2 may explain the heightened ammonia production (see Hobson<sup>6</sup>).

The source of the ammonia is not definitely known. Hobson<sup>6</sup> believed that it resulted from the deamination of the amino groups set free as the maggot hydrolyzed its food, but was unable to identify a deaminase in his study of the enzymes of blowfly larvae. Nitrogen distribution studies, still incomplete, have convinced us that Hobson's belief is correct.

#### THE MECHANISM FOR THE REMOVAL OF NECROTIC TISSUE

All who have watched osteomyelitis wounds healing under treatment with blowfly larvae are impressed with the rapid cleansing of the wound and the resulting pinkish granulation tissue and flat, undistorted scar. Baer, the first proponent of larval therapy, ascribed such results to what he called the "scavenger action" of the maggots;<sup>1</sup> their peculiar food requirements permitting them to attack only dead tissue.

Fabre<sup>3</sup> described the way in which maggots feed upon meat; first liquefying the protein with a proteolytic saliva or excretion, after which they ingest and assimilate the liquid food. Hobson has carried out an investigation of the digestive enzymes of *Lucilia sericata* larvae, and has found a trypsinlike protease in both the digestive tract and excreta,<sup>6</sup> and a collagenase in the excreta.<sup>7</sup> In England, this larva is said to infest sheep, boring into the flesh through the skin. The American variety of the same species, however, was chosen for surgical purposes, because here it is known never to attack living tissue. We have studied the digestive enzymes of *L. sericata* to learn if such a difference in food habits involved a corresponding difference in enzyme mechanism.

Lots of about 500 sterile, half-grown larvae were ground with sand and 50 c.c. of solvent. The ground mixtures were incubated for varying lengths of time at 40° C. and filtered. The proteolytic activities of these extracts were ob-

ferent times of day and night, it was found necessary to include in the line a mercury valve, *G*, and a five-gallon carboy, *H*, which acted as an air reservoir. A similar train, containing a culture bottle, but without eggs, was placed in parallel as a control ( $A_1, \dots G_1$ ).

At the end of the chosen period both the bottle containing the maggots and the control bottle were removed from the train and acidified with a few cubic centimeters of 0.1 N hydrochloric acid. The maggots were removed by means of forceps, washed, dried on filter paper, counted, and weighed as a group. The washings were added to the culture bottle, which was heated on a water-bath to melt the agar and diluted to 250 c.c. The control bottle was similarly treated. The standard acid in the absorption tubes was titrated, and ammonia nitrogen determined on 25 c.c. aliquots of the solution of the maggot medium and of the control medium, using Saehsse's method<sup>13</sup> of vacuum distillation in the presence of an excess of magnesia.

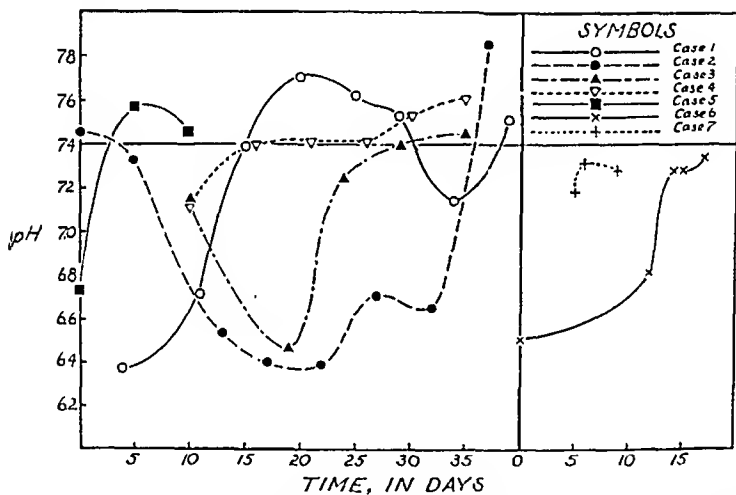


Fig. 2.—Variations in the pH of wound exudates. Cases 1 to 5, chronic osteomyelitis treated with blowfly maggots. Case 6, appendiceal abscess. Case 7, compound fracture of the tibia. Wet dressings of physiologic salt solution only were used in Cases 6 and 7.

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actuated by the motor of a pneumatic windshield wiper. At the end of the seven-hour period, the maggots were all living and vigorous.

The fluid in which the maggots had been immersed more than 1,500 times over a seven-hour period was tested for proteolytic activity against peptone and gelatin, using the same technique as previously described. For comparison, a 1 per cent solution of Armour's trypsin was tested simultaneously. As Fig. 4 shows, the washings, in volume comparable to the extracts described above, showed a tryptic activity barely beyond the limits of error of the method, whereas the trypsin preparation is comparable in potency to the extracts of crushed maggots.

We were not able to identify a collagenase in the solution of excreta, although Hobson appears to have found one in that variety of *L. sericata* common in England. Since such an enzyme would aid the larva to penetrate through the intact skin of an animal, we believe that this difference in enzyme equipment is a reflection of the difference in feeding habits between the English variety of *L. sericata* and that found in America.

We believe our observation that blowfly maggots excrete a weakly proteolytic fluid, while containing in their digestive tracts a relatively strong one, explains why they remove necrotic tissue as effectively as they do. The use in a wound of an enzyme solution sufficiently powerful to hydrolyze the dead tissue completely would be highly irritating. The maggots apparently excrete a fluid just sufficiently proteolytic to liquefy their food, while the bulk of the digestion takes place within their digestive tracts, out of contact with the tissues of the wound. Another important factor in the "scavenger action" is that the proteolytic end-products, which would otherwise be absorbed by the patient's system, or remain in the wound to furnish a substrate for bacteria, are used by the maggots for growth, and thus rendered inert.

#### DISCUSSION

It is interesting to note that both the alkalization of the wound and the removal of necrotic tissue by maggots depend on the presence of living larvae. Similarly, Robinson and Norwood<sup>11</sup> have shown that maggots are capable of ingesting and destroying by digestion large numbers of bacteria. These facts demonstrate the impossibility of substituting for the living organism extracts or pastes prepared from blowfly larvae, as has been suggested in the past.<sup>5, 10</sup>

Indeed, it is reasonable to suppose that much of the unique virtue of maggot therapy depends upon minuteness and thoroughness of action, and the uniformity with which the concentration of any active agent which the larvae may excrete may be maintained in the wound. Compared with these conditions, which are best realized in the presence of large numbers of minute active agents, surgical instruments and bactericidal dressings are relatively crude tools.

#### SUMMARY AND CONCLUSIONS

1. Chronic osteomyelitis wounds, healing in the presence of blowfly larvae, develop reactions more alkaline than pH 7.4, in contrast to wounds dressed only with physiologic salt solution.

2. It has been shown that sterile *Lucilia sericata* larvae produce sufficient ammonia to account for this excess alkalinity.

served toward 10 per cent solutions of casein, gelatin, and peptone, buffered to a pH of 8.0. A modification of the method of Willstätter and Grassman<sup>14</sup> was used to follow the digestion. Sufficient alcohol was added to aliquots of the digest to make 90 per cent alcoholic solutions. They were then titrated with 0.05 N potassium hydroxide in 90 per cent alcohol, from a microburet, using phenolphthalein as an indicator. Under these circumstances, the carboxyl groups freed by proteolysis dissociate, while the corresponding amino groups do not; consequently, the progressive increase in titrable acidity is a measure of proteolysis. Since heating would alter the base-binding capacity of both substrate and enzyme solution, the controls were not heat inactivated. Instead, controls consisting of substrate, and of extract, were incubated in separate tubes, and samples of each titrated along with those of the digestion mixture. From the titration value of the sample of digestion mixture was subtracted the sum of the values for both controls, and the difference gave a figure which was proportional to the degree of proteolysis.

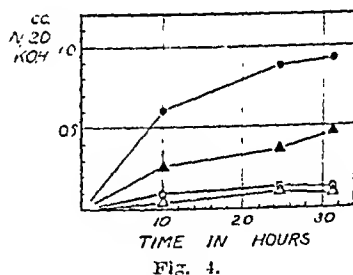
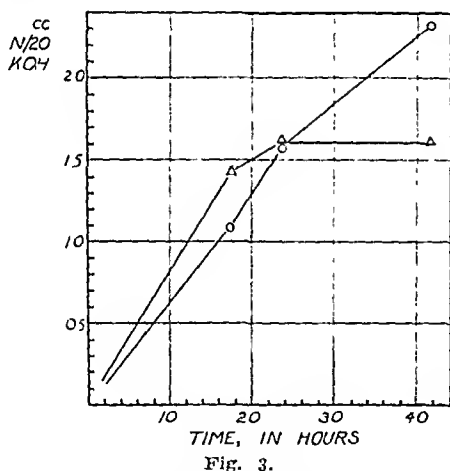


Fig. 3.—Proteolytic activity of a 20 per cent glycerol extract of maggots. Ordinates, amounts of KOH necessary to neutralize carboxyl groups liberated. Circles, casein; triangles, peptone.

Fig. 4.—Proteolytic activity of maggot washings compared with that of 1 per cent trypsin. Solid circles, trypsin against gelatin; solid triangles, trypsin against peptone. Hollow circles, washings against gelatin; hollow triangles, washings against peptone.

The best medium for extracting the proteolytic enzymes was found to be 20 per cent glycerol. Eighty per cent glycerol was an inferior solvent, while 0.9 per cent sodium chloride extracted only a trace of active material.

Fig. 3 represents the results of a typical experiment in which crushed maggots were incubated with 20 per cent glycerol for twenty-four hours. By comparison with the digestion rate of Armour's trypsin in Fig. 4, it will be seen that this extract possessed a high proteolytic potency.

In order to obtain a solution of the excretory products of the larvae, we confined lots of maggots in a cylindrical cage of 60 mesh copper gauze and dipped them repeatedly into physiologic salt solution. The cage, containing 200 to 300 maggots, was raised and lowered into 50 c.c. of the salt solution four times per minute for seven hours, by means of a cord passing over pulleys, and

# THE PRIMARY CARCINOMA OF THE LUNG\*

## A REVIEW OF 100 AUTOPSIES

R. H. JARRÉ, M.D., CHICAGO, ILL.

IN RECENT years numerous reports have appeared from different parts of the world which deal with the increase in frequency of the primary carcinoma of the lung. Formerly considered a rare disease, seldom correctly diagnosed during life, modern statistics give the incidence of primary carcinoma of the lung with from 8 to 20 per cent of all carcinomas. Much controversy has arisen as to the cause of this high incidence of pulmonary carcinoma compared with the incidence of two or three decades ago. Many authors emphasize the great importance of the persistent irritation of the lungs by dust and gases which are the result of modern life and which are known to contain carcinogenic substances. Thus, by tar, pulmonary carcinoma has been produced experimentally in mice. Carcinoma of the lung, however, is also very common in countries in which the tarring of roads is but little used and automobiles are few in number (e.g., Russia). Other investigators stress, therefore, the significance of pulmonary infections, in particular the last influenza pandemic and focal, chronic pneumonias which seem to be more common than in former years. On the other hand, there is a group of authors who believe that the increase in frequency of pulmonary carcinoma is merely due to the fact that we have learned to recognize it better, and that with the increasing span of life more people reach the age in which carcinoma of the lung is most common.

Whether its increase in frequency is real or apparent, carcinoma of the lung, undoubtedly, is a common disease and a thorough familiarity with it is of importance. The great progress in thoracic surgery and in radiotherapy makes one hopeful that, if diagnosed early, pulmonary carcinoma is not an absolutely fatal disease, and an increasing number of reports are being published which suggest a complete cure or, at least, considerable prolongation of life by surgery or radiation. The early diagnosis of a disease is intimately linked to the knowledge of its anatomical manifestations. The modern literature contains many studies on the pathology and histogenesis of pulmonary carcinoma and, in addition to the chapters in the textbooks on malignant neoplasms (Ewing, Stout), I refer to the monographs and articles respectively of W. Fischer, Fried, Hruby and Sweany, Huguenin and Weller. Personal experience with a large number of cases has the advantage of uniform analysis and justifies the publication of additional material. It is with this point of view in mind that I present the following review of one hundred cases of primary carcinoma of the lung which have come under my observation during the past six years and which have been proved at autopsy and by microscopic examination.

\*From the Department of Pathology of the Cook County Hospital.  
Received for publication, March 15, 1934.

3. The excess alkalinity is probably a factor in bacteriostasis and wound healing.

4. Blowfly larvae are shown to excrete a relatively weak proteolytic enzyme, while they contain in their digestive tract a more powerful one.

5. The relative strength and location of these enzymes permit the removal of necrotic tissue from a wound with a minimum of irritation.

6. The assimilation by the larvae of the protein split products of necrotic tissue removes the latter from the wound where they would otherwise putrefy or be absorbed, to the detriment of the patient.

7. Maggot therapy depends for its beneficial action on the presence of living larvae, which cannot be successfully replaced by pastes or extracts of maggots.

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NOTE: Since the above was written, Dr. William Robinson (*J. Bone & Joint Surg.* 17: 267, 1935) has pointed out that allantoin, present in the excreta of maggots, stimulates cell proliferation, and recommends its use to aid the healing of indolent wounds and ulcers. If such treatment proves successful, one function of maggot therapy, the stimulation of rapid healing, can be carried out in the absence of living larvae by means of a larval catabolite. We affirm our last conclusion, however, with respect to the two functions of maggot therapy which we have particularly studied: the removal of necrotic tissue, and whatever degree of bacteriostasis may be effected.

*Age, Sex and Race.*—All statistics agree that carcinoma of the lung is most common in the fifth decade of life and that it is much more frequent among males than among females. According to the age my hundred cases are distributed as follows:

Below 30 years (youngest case 24 years)	2
31-40 years	6
41-50 years	30
51-60 years	41
61-70 years	16
Over 70 years	5

Ninety-two per cent were in males. Racial differences were not observed. The total incidence of pulmonary carcinoma was higher in the white race than in the colored race, namely, 1.7 per cent of all autopsies in the white race and 1.0 per cent of all autopsies in the colored race. This difference is of no significance because the average span of life of my material is about ten years lower in the colored race than in the white race. In the colored race the difference between males and females was less striking than in the white race, 35 per cent of the tumors being found in negroes.

*Location.*—In most of the statistics the right lung has been found to be more frequently involved than the left lung. In some statistics the opposite is the case (e.g., Kraft, Edwards). In my material the relation between carcinoma of the right and left lung is 55:45. Fifty-five tumors involved the upper lobes and 33 tumors involved the lower lobes. There was only one carcinoma of the right middle lobe. In eleven cases the tumor had originated from the main bronchus proximal to its first division.

*Macroscopic Appearance.*—The gross appearance of the tumor depends chiefly upon the extent of the local progression. If local complications or the metastases cause the patient's death at a relatively early stage of the disease, the primary neoplasm in the lung may be so small that it is easily overlooked at autopsy. In these cases, one finds, in a larger or smaller bronchus, a circumscribed, indurated area of the mucosa with or without slight infiltration of the surrounding lung tissue. In some cases a small and friable polyp may protrude into the lumen of a bronchus. If such a polyp has become sequestered and expectorated, there may be a small and shallow ulcer, the true nature of which can be recognized only on histologic examination. More frequently, the tumor has grown to a more or less circumscribed nodule or to a large node surrounding a bronchus and compressing its lumen. There are cases of pulmonary carcinoma which present themselves as diffuse consolidations of part of a lobe or of an entire lobe, resembling a lobular or lobar pneumonia. In some instances regressive changes have transformed the tumor into a huge cavity with a thin lining of intact tumor tissue. Most carcinomas of the lung are soft, medullary in consistency and pale gray or grayish white in color.

For practical purposes Rabin and Neuhoof distinguish two main groups of cancer of the lung. One group is called the noncircumscribed type. This type to which three-fourths of the tumors belong originates from the main bronchus



*Frequency.*—My material consists of 6,800 autopsies, 871 of which have been on cases with carcinoma. With *11.47 per cent* carcinoma of the lung is third in frequency among the carcinomas, carcinoma of the stomach being first with 184 cases, and carcinoma of the intestine being second with 118 cases. In 1921, I published, from a different part of the world (Vienna, Austria), statistics which were based upon 4,500 autopsies performed between 1915 and 1918. In these statistics the incidence of pulmonary carcinoma among the carcinomas was 10.73 per cent. It is, of course, not permissible to compare figures which have been collected under entirely different conditions, but the similarity between the two figures is so striking that I have thought them worth while quoting. I am inclined, therefore, to believe with Fried and others that the increase in frequency of primary carcinoma of the lung is more apparent than real.

*Etiology.*—My material does not offer any definite suggestion as to the probable etiology of pulmonary carcinoma. The patients came from the most different walks of life. Fine and Jaso and others assume that silicosis may predispose to pulmonary carcinoma. Among my hundred cases there was only one with the anatomic evidences of silicosis and in this case the silicon content of the lung amounted to 4.79 mg. per one gram dried lung tissue. Allen believes that in the coal miners of Pennsylvania carcinoma of the lung is not more common than in the other population. In a critical review Saupe comes to the conclusion that the high incidence of pulmonary carcinoma in certain mining districts is due rather to radioactive substances in the air of these mines than to anorganic dust. The tendency of syphilis to cause chronic irritation of the mucous membranes with epithelial metaplasia and carcinomatous transformation of the metaplastic epithelium has often been used as an argument that syphilis may play an important rôle in the pathogenesis of carcinoma of the lung. Twenty per cent of my cases of carcinoma of the lung showed anatomic or serologic evidences of syphilis which is distinctly higher than in the rest of my material of individuals over twenty years of age (approximately 11 per cent). L. Popper states that 12 per cent of his cases of bronchiogenic carcinoma have shown evidences of syphilis while in 337 cases of other carcinomas this combination has been found in only 3.9 per cent. In seven instances I have found, in addition to the carcinoma, a progressing tuberculosis of the lungs. The anatomic findings suggested that the carcinoma had stimulated to active progression a silent preexistent tuberculosis. There was no case of carcinoma formation from the metaplastic epithelium of an old tuberculous cavity.

None of my cases showed definite relations to a preceding trauma. There was one case of carcinoma of the lung in a colored man, forty-three years of age, who, several years prior to his death, had received a bullet wound to the chest. At autopsy the bullet was found, well encapsulated, in the right lower pulmonary lobe, while the left lung showed a squamous cell carcinoma that had originated from the main bronchus. I think that these findings exclude the possibility that the old injury may have been related to the tumor. In general, it may be said that in the majority of the cases, at the time of death, the tumor has advanced so far as to obscure any preceding or predisposing local changes.

thelial cells appeared very irregular with hyperchromatic, anaplastic nuclei and atypical mitotic figures. In one area there was a distinct invasion of the connective tissue surrounding the bronchus and lymph vessels and small veins were found filled with the atypical cells (Fig. 2). In another case in which the patient died from a hemorrhage into a cerebral metastasis a small primary tumor was found in the apex of the left upper pulmonary lobe.\* With its necrotic center the primary tumor resembled somewhat an apical tubercle except for the medullary, grayish white tissue about the necrosis. The histologic examination disclosed a very immature round-celled carcinoma. The tumor cells were arranged in small alveoli. According to my experience the peripheral carcinoma offers the greatest diagnostic difficulties. It may spread diffusely over the pleural surface of the



Fig. 2.—This area shows the invasion of the stroma by the atypical cells, some of which are found in the lumen of lymph vessels. The cells also extend into and line the two alveoli seen at the left and right side of the field. Staining and magnification same as Fig. 1.

whole lobe or over the entire lung encasing it into a rousseau of tumor tissue. These cases are often mistaken for mesotheliomas of the pleura, a tumor which is exceedingly rare. In the central type of pulmonary carcinoma this diffuse pleural spreading is less common. In six cases the tumor was located in the center of a lobe originating from a bronchus of third, fourth, or fifth order (intermediary type). Finally, there were five cases with diffuse involvement of a lobe.

*Associated Changes in the Lung.*—In many instances the carcinoma leads to secondary changes which may be restricted to the lobe that is the site of the

\*According to the observations of Steiner and Franel the "superior pulmonary sulcus tumor" described by Pancoast corresponds most likely to an apical carcinoma of the lung.

or from a branch bronchus and reveals the usual invasive characteristics of cancer. The second group is called the circumscribed type and presents itself as a spherical, well-demarcated growth macroscopically unrelated to a bronchus. Cancers of the circumscribed type occupy the parenchymal and peripheral zones (parenchymal and peripheral tumors). Geschiekter and Denison also refer to two groups and speak of a hilar or epidermoid carcinoma that is most common and occurs at the center of the lung, and of a lobular carcinoma that arises in the periphery of the lung. I have divided my material of pulmonary carcinoma into four types, namely, the central or hilus, the intermediary, the peripheral and the diffuse type. The central type, which starts from the main bronchus or its first divisions and quickly spreads to the hilus and mediastinal lymph nodes



Fig. 1.—Peripheral, chronic interstitial pneumonia. In the center of the field is a small bronchus which is lined by metaplastic epithelium. There is some irregularity in the arrangement and shape of these cells. The lumen of the bronchus contains clumps of pus cells. Mallory's phosphotungstate hematoxylin stain, magnification  $\times 150$ .

fusing with them to huge solid masses, is the most common type. It was found in 76 per cent of my cases. With 13 per cent the peripheral type is next in frequency. It starts in the periphery of the lung underneath the visceral pleura. In a case which is not included in my statistical figures a peripheral carcinoma was discovered at autopsy as an incidental finding. The patient was a white man, fifty-nine years of age, who for many years, in spite of all treatments, had a persistently strongly positive Wassermann reaction and who died from decompensation of a hypertrophic heart. The lower border of the right lower lobe felt firm and nodular. On microscopic examination there was a marginal, chronic, interstitial pneumonia with extensive metaplasia of the epithelium of the smallest bronchi into squamous epithelium (Fig. 1). In several places the metaplastic epi-

two of my hundred cases no metastases were found at autopsy. The other cases showed the following distribution of the metastases:

Thoracic lymph nodes	89 times	Pancreas	6 times
Other lung	43 times	Spleen	5 times
Adrenals	42 times	Peritoneum	4 times
Abdominal lymph nodes	37 times	Dura mater of spinal cord	3 times
Liver	36 times	Skin, thyroid, pericardium, muscles	2 times
Kidneys	28 times	Stomach, seminal vesicles, tongue, ovary, testicle,	
Bones	22 times	breast	1 time
Brain	19 times		
Intestine	8 times		
Heart	7 times		

The figures for the metastases to the brain and bones are apparently too low, since in twenty-three cases no permission was obtained for removing the skull, and a systematic examination of the bones could not be made. Schmorl points out that in 33.3 per cent of the cases with pulmonary carcinoma metastases can be found in the spine.

*Histopathology.*—As source of the primary epithelial neoplasm of the lung the surface epithelium of the bronchi, the epithelium of the mucous glands of the bronchi and the alveolar epithelium have been considered. As far as the alveolar epithelium is concerned it appears doubtful whether the lung of adult human beings possesses an epithelial lining. The majority of the modern investigators trace the pulmonary carcinoma chiefly to the bronchi (Kraft, Sweany, Fried, Fischer, Tuttle and Womack and others). They are, particularly, the short, dark nucleated epithelial cells which are wedged in between the basal portion of the ciliated epithelial cells and which have been designated as basal cells from which the carcinoma of the lung seems to originate. These basal epithelial cells supply the material for epithelial regeneration and are endowed with the prosoplastic potentialities to differentiate into ciliated or nonciliated columnar cells, mucus-producing epithelial cells and squamous epithelial cells. The carcinoma of the lung shows great variations in the microscopic structure and different areas from the same tumor may reveal widely different pictures.

In thirty-one of my cases the tumor was composed of undifferentiated, round cells with scanty cytoplasm and round nuclei rich in chromatin. The cells varied from six to twelve microns in diameter and formed solid areas which were surrounded by a scanty stroma with thin-walled capillaries. In the center of the solid areas necrosis was common. This type of tumor has been called round-celled or solid carcinoma, and its resemblance to sarcoma has often been stressed. In 28 cases the tumor cells were distinctly larger and more pleomorphic and possessed an ample cytoplasm. The shape was polygonal, elongated, or oval, and the nuclei contained a compact chromatin net and prominent nucleoli. In some of the tumors huge giant cells were encountered. These cells arranged themselves in a pavement-like fashion resembling closely the undifferentiated squamous cell carcinoma of the mucous membranes. In Fischer's statistics too, type of carcinoma of the lung as pleomorphic celled (Kraft and others). Among the squamous cell carcinomas there were three with hornification and numerous

tumor or which may involve the entire lung. These secondary changes are, sometimes, so marked that they obscure the tumor. In thirty-six cases the carcinoma of the lung was associated with diffuse or saccular bronchiectases and the relation between the tumor and the bronchiectases indicated that the widening of the bronchial lumen was secondary to the tumor stenosis. In only one case did the carcinoma originate from the epithelial lining of an old bronchiectatic cavity. There were 17 cases of chronic pneumonia distal to the tumor, 8 cases of lung abscess and 4 cases of lung gangrene. The abscesses and gangrenous areas involved portions of the lung peripherally to the tumor.

*Changes of the Pleural Cavity.*—Spreading along the peribronchial lymph vessels the carcinoma often reaches the pleural cover causing reactive changes of the pleura. Recent exudative processes were encountered in 43 of my cases. The character of the exudate was as follows: serous, 14 cases; hemorrhagic, 13 cases; suppurative, 12 cases; fibrinous, 4 cases. There were 26 cases of partial and 23 cases of complete obliteration of the pleural cavity. In 8 cases the pleura did not show any changes.

*Compression and Invasion of Adjacent Structures.*—It is particularly the hilus type of pulmonary carcinoma with its tendency to spread toward the mediastinum which leads to the involvement of the neighboring organs. In seven cases the tumor had invaded and obliterated the superior vena cava, causing the formation of extensive collaterals in the anterior wall of the chest. In two cases the inferior vena cava was found obliterated by tumor tissue. There was one case each of invasion of the trachea and esophagus and three cases of direct extension of the tumor through the pericardial sac into the myocardium of the right auricle. Compression of the trachea was encountered twice and compression of the esophagus four times. In all these cases the metastases to the tracheobronchial and mediastinal lymph nodes had fused with the pulmonary tumor into a solid mass. There were four instances of erosion of a larger pulmonary vein with severe hemorrhage. In one case the pulmonary tumor had grown through the foramina intervertebralia into the spinal canal compressing the midportion of the dorsal cord. One peripheral carcinoma had extended through the intercostal spaces into the pectoral muscle impressing, at first, as a sarcoma of the major pectoral muscle.

*Metastases.*—The carcinoma of the lung is characterized by a most extensive hematogenous and lymphatic spreading. It has already been mentioned that an insignificant primary tumor may be found in cases of generalized metastases. Because the pulmonary carcinoma sometimes presents itself under the picture of a primary tumor of the brain, French authors refer to a cerebral form of carcinoma of the lung. With the same justification one could speak of a gastric, intestinal, osseous or lymph node form of the pulmonary carcinoma. Compression of the duodenum by metastases to the peripancreatic lymph nodes, or compression of the cardia of the stomach by metastases to the perigastric lymph nodes, or compression of the common duct by metastases to the peribiliary lymph nodes often directs the clinician's attention to this region of the body. In only

of confusion. On admission 54 patients complained chiefly of weakness and loss of weight. The loss of weight varied between 10 and 60 pounds. Pain in the chest was encountered 34 times and was often described as sharp and shooting. In 54 cases a persistent cough was among the first symptoms, and in 22 cases the cough was said to be productive of a blood-streaked material. Twenty-eight patients referred to shortness of breath and 5 patients complained of difficulties in swallowing. There were 3 cases in which hoarseness was the first symptom. Twenty-four patients showed no subjective symptoms pointing toward the chest. Fourteen patients complained of abdominal or epigastric pain, 10 patients of loss of appetite, nausea, and vomiting. Five patients referred to constipation. Hess and Faltisehek have discussed persistent constipation in cases of bronchial carcinoma. This constipation affects mainly the proximal portion of the colon and has been attributed to the involvement of the right vagus by a hilus tumor of the right lung. In 13 cases the symptoms pointed to the central nervous system.

*Diagnosis.*—My study is based upon the material of a large charity hospital and many patients enter the hospital in an advanced stage of the disease. Their average stay in the hospital is short and language difficulties are often encountered. Some of the patients with pulmonary carcinoma were too ill for a thorough clinical, bronchoscopic or roentgenologic examination. The longer the stay in the hospital the higher also was the percentage of correct diagnoses. Taking all cases together 59 were correctly diagnosed as pulmonary carcinoma. In the first three years 47.5 per cent were missed while in the last three years, after many cases had been shown in the pathologic-anatomic demonstrations, the percentage of missed diagnoses dropped below 30 per cent. The highest incidence of incorrect diagnosis was found in the peripheral type of pulmonary carcinoma, namely 46 per cent. Of the incorrect diagnoses carcinoma of the stomach is of particular interest. This diagnosis was made in nine cases of carcinoma of the lung. In these cases extensive metastases were found in the perigastric or peripancreatic lymph nodes, compressing the cardiac or pyloric portion of the stomach or the duodenum. In six cases the metastases had perforated into the lumen of the stomach or duodenum, causing confusing x-ray findings and the presence of occult blood in the feces. The other incorrect diagnoses were:

Pulmonary tuberculosis	9 cases
(Five of these cases showed tubercle bacilli in the sputum and were found, at autopsy, to be combined with active tuberculosis)	
Lung abscess	3 cases
Decompensated heart	1 case
Brain tumor or central nervous system syphilis	6 cases
Sarcoma of bone (metastasis to sternum)	1 case
Carcinoma of breast (metastasis to breast)	1 case
Hodgkin's disease	1 case
Allostatic lymphadenosis	1 case
In these two cases there were generalized metastases to the peripheral lymph nodes and patients refused the taking of biopsies	
Carcinoma of the prostate	2 cases

horn pearls. Since the basal cells are potentially able to differentiate into squamous epithelium, the high incidence of squamous cell carcinomas of the lung is not surprising. Some cases suggest, however, that the squamous cell carcinoma may develop from a previously metaplastic epithelium. I have repeatedly seen in cases of squamous cell carcinoma of the lung areas of metaplasia of the bronchial epithelium far away from the tumor. There were twenty-four instances of adenocarcinoma in three of which the formation of papillae was an outstanding feature. Whether the tumor glands are derived from the surface epithelium or from the epithelium of the mucus gland I cannot decide. In three cases of mucus-producing carcinoma an origin from the goblet cells of the surface epithelium or from the mucus glands may be considered. The type of pulmonary carcinoma which is characterized by the spindle shape of the cells and which, under the term "oat celled" carcinoma, has been discussed especially by British authors (Barnard and others), has been encountered eight times. The types of tumors discussed so far did not differ in their gross appearance nor were there any differences in the duration of life or the extent of metastases.\* In four cases the tumor was firmer and on microscopic examination small alveoli of cuboidal or cylindric cells were found which were embedded by an ample stroma. These cases were called carcinoma simplex. In two cases in which the primary tumor was very small and appeared as a firm, circumscribed thickening of the bronchial mucosa, the histologic picture was that of a scirrhus. Both carcinoma simplex and scirrhus had produced extensive metastases.

*Duration.*—In a disease in which the onset is as insidious as in pulmonary carcinoma, it is impossible to determine the duration of life after the tumor has started to develop. The following table is based upon the appearance of the first symptoms which are probably due to the pulmonary lesion. Seven cases are excluded because the patients died soon after admission without adequate history.

Less than 1 month	3 cases
1 to 3 months	30 cases
4 to 6 months	24 cases
7 to 12 months	26 cases
Over 1 year	7 cases
Over 2 years	3 cases

In fifty-seven cases the duration of the illness was less than six months while in only ten cases did the patient live a year or longer. I did not find any definite relation between the size of the primary tumor or the extent of the metastases and the duration of the illness. From the statistics of Tuttle and Womack it appears that the average duration of life is shorter in the cases with the peripheral type of pulmonary carcinoma than in the cases in which the tumor originates in major bronchi. In my material the average duration of life was eight months in the central type and five months in the peripheral type.

*First Symptoms.*—It is not the purpose of this paper to enter into a discussion of the clinical aspects of pulmonary carcinoma. A summary of the first symptoms, however, may be given since I have found them a frequent source

\*I fully agree with Tuttle and Womack and others that the grading of bronchiogenic carcinoma on histologic grounds is of little practical value.

recalled that Hodgkin's disease of the lung may so closely resemble pulmonary carcinoma that differentiation is possible only by histologic examination.

### SUMMARY

The autopsy findings of one hundred cases of primary carcinoma of the lung are reviewed. Except for a high incidence of syphilis no suggestive etiologic factor was recorded. The great predominance of the male sex was confirmed. Racial differences were not observed. Whether carcinoma of the lung is really increasing in frequency appears doubtful.

Macroscopically a central, intermediary, peripheral and diffuse type of pulmonary carcinoma have been distinguished. The peripheral type seems to offer the greatest diagnostic difficulty.

The majority of the pulmonary carcinomas are composed of undifferentiated round cells which are apparently derived from the basal cells of the bronchi. The high incidence of squamous cell carcinoma suggests relations to epithelial metaplasia. Other types of pulmonary carcinoma are: the adenocarcinoma, the mucus carcinoma, the carcinoma simplex, and the scirrhous.

The differential diagnostic difficulties which are due to the early appearance of metastases to distant organs are discussed. The value of biopsies is emphasized.

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In seven cases no diagnosis was made because the patient died soon after admission.

In this connection it may be mentioned that extrapulmonary carcinomas may present the clinical and also the anatomic picture of a primary carcinoma of the lung. Thus, I observed two cases of carcinoma of the prostate which, at autopsy, were thought to be carcinomas of the lung. In these two cases there was a single large metastasis to the lung which had perforated into an adjacent bronchus and branch of the pulmonary vein with extensive secondary, hematogenous dissemination to many organs, especially to the adrenals, kidneys, and brain. The prostate was slightly enlarged and soft but on microscopic examination a typical carcinoma of the prostate was discovered.

*Value of Biopsy in Pulmonary Carcinoma.*—Tumors of the large bronchi which can be seen on bronchoscopic examination lend themselves to biopsies. According to C. L. Jackson bronchoscopic biopsy will be positive in about 75 per cent of the cases of bronchiogenic carcinoma. In taking bronchoscopic biopsies one has to be careful to select the proper area, avoiding necrotic tissue and noncharacteristic reactive changes peripherally to the tumor. Without positive bronchoscopic findings there are other possibilities to confirm the diagnosis by a biopsy. I am referring especially to the high incidence of metastases to peripheral lymph nodes. In twenty-eight of my cases enlarged lymph nodes could be palpated during life. The following lymph nodes were found to be involved:

Cervical and supraclavicular	26 times
Submaxillary	3 times
Axillary	4 times
Inguinal	2 times

Sputum and pleural effusion yield valuable material for histologic examination. They can be precipitated and fixed with alcohol and embedded with paraffin. In two of my cases with skin metastases biopsies from the skin directed the clinician's attention to the lung. Finally biopsies from bone metastases and from the lung and pleura with the aid of an exploratory thoracotomy may be mentioned.

*Extrapulmonary Complications.*—Eight cases of pulmonary carcinoma showed, at autopsy, an exudative pericarditis. In three cases a terminal endocarditis was found. There were two cases of purpura hemorrhagica and two cases of severe jaundice due to the compression of the common duct by metastases to the peribiliary lymph nodes. There was one case of amyloidosis and only one case of hypertrophic osteo-arthritis (Marie-Bamberger). Four cases showed a very marked clubbing of the fingers. In ten cases the spleen was palpable during life.

*Other Primary Tumors of the Lung.*—Apart from carcinoma primary tumors of the lung were very rare in my material. There was one case of benign, glandular polyp and two cases of chondroma, one of which had developed in the wall of a bronchiectatic cavity. There was one case of mesothelioma of the pleura but no case of true sarcoma of the lung. In six of thirty cases of Hodgkin's disease the lungs were found to be extensively involved. It may be

of horse serum. Upon repeated intravenous injections, occasional reactions, usually general in character, occurred. One dog, however (No. 51, sac without

TABLE I  
RESULTS OF INTRAVENOUS INJECTION OF HORSE SERUM

DOG. NO.		HORSE SERUM TREATED								CONTROLS					
		52		62		53		70		51		63		73	
PREPARATION		HIS SAC		HIS SAC INJURY		HIS IN-JECTED		HIS IN-JECTED		SALINE SAC		SALINE SAC INJURY		SALINE INJECTED	
NO. WEEKS AFTER PREPARATION	ANTI-OEN I. V.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.
3	HIS	++++	++++							0	0				
7	HS			*+	++++							0	0		
8	HIS					0	0	0	0					0	0
11	HS			++	0							++++			
12	HS									0	0				
17	HS					0	0	++	+++					0	0
21	HIS			+	0					++	++				
26	EW					0	0	0	0						
27	HS					++	0	+	0					0	0
29	EW									0	0				
30	HS			0	0					++	0				
37						0	0	+++	0						
39				0	0					+	0				

\*Delayed reaction, four hours after I.V. injection. EW, Egg white. Loc., Local reaction. HS, Horse serum. Gen., General reaction.

TABLE II  
RESULTS OF INTRAVENOUS INJECTION OF EGG WHITE

DOG NO.		EGG TREATED				CONTROLS					
		57		68		58		64		72	
PREPARATION		EW SAC		EW INJECTED		SALINE SAC		SALINE SAC INJURY		SALINE INJECTED	
NO. WEEKS AFTER PREPARATION	ANTIGEN I. V.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.
7	EW	0	0			0	0	0	0		
8	EW			++	0						
9	EW									0	0
11	EW							0	0		
12	EW	0	0			++	0				
18	EW			+++	0					0	0
21		++	0			+++	+++	++	0		
27				+++	0					0	0
30		+++	0			+	0	++	0		
35				+	0						
39		+	0			+	0	0	0		

injury), exhibited a slight focal reaction after the third intravenous injection of the antigen. Although in the initial operative procedure the cerebrum was not purposely injured, undoubtedly a certain degree of trauma occurred in the

### III. LOCAL CEREBRAL ANAPHYLAXIS IN THE DOG\*

LEO M. DAVIDOFF, M.D., NICHOLAS KOPELOFF, PH.D., AND LENORE M. KOPELOFF,  
M.A., NEW YORK, N. Y.

IN ORDER to augment the material presented in previous communications<sup>1</sup> and particularly to increase the number of control animals, a further study of twenty-three dogs was undertaken. These dogs varied in weight between 4 and 14 kg., the average being 8 kg. Two methods of sensitization were employed. The first consisted in placing the antigen in a small collodion sac in contact with the cerebrum, which was either purposely injured by scarification or left comparatively intact. In control animals the sacs were filled with sterile physiologic saline. In the second method, on five successive days 0.2 c.c. of antigen was injected directly into the brain through a previously prepared trephine hole after allowing the wound to heal. Control animals received similar injections of saline.

The two antigens used were normal horse serum,<sup>†</sup> which was always employed on the left side of the brain, and fresh egg white, which was always used on the right side of the brain. The sacs were filled with 0.05 c.c. of equal parts of horse serum and saline, or 0.10 c.c. of 17 per cent egg white (diluted with saline). The test for sensitivity consisted of an injection into the leg vein of 4 c.c. of undiluted normal horse serum or egg white.

In Tables I to III the values assigned to the general reaction represent degrees in terms of the number of responses noted, i.e., diarrhea, vomiting, weakness, salivation, and death. The degree of intensity of the focal reaction is indicated as very slight ( $\pm$ ), slight (+), moderate (++), marked (+++), and very marked (++++). It goes without saying that any focal reaction observed occurred on the side of the animal opposite the side of the brain which was in immediate contact with the antigen.

#### HORSE SERUM

Considering first the dogs which received horse serum alone, we found that after the first intravenous injection (No. 52, sac without cerebral injury), there occurred a very marked contralateral paralysis accompanied by an extremely severe general reaction terminating in death one-half hour after the injection of horse serum. Another dog (No. 62, sac with injury) did not react immediately to its first intravenous injection of horse serum, but three hours later salivated, rotated its head to the right, showed convulsive twitching of the right forepaw, and backward stamping of the right hind leg. Control dogs (saline sac in contact with the brain) did not react in any way to the first intravenous dose

\*From the Department of Bacteriology, New York State Psychiatric Institute and Hospital.

†This was very generously placed at our disposal by the New York City Board of Health Research Laboratories.

Received for publication, March 27, 1935.

TABLE III

RESULTS OF INTRAVENOUS INJECTIONS OF HORSE SERUM AND EGG WHITE

DOG NO.		HS AND EW TREATED										CONTROLS			
		54		66		55		65		67		61		71	
PREPARATION		HS SAC EW SAC		HS SAC EW SAC		HS SAC EW SAC INJURY		HS EW INJECTED		HS EW INJECTED		SALINE SAC INJURY		SALINE INJECTED	
NO. WEEKS AFTER PREPA- RATION	ANTI- GEN I. V.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.	GEN.	LOC.
3	HS	+++	±			+++	0								
7	HS											0	0		
	EW	++	0			0	0					0	0		
8	HS							+	0	+++	0			0	0
9	EW							0	0	+	+++			0	0
10	HS			0	0										
11	HS			±	0							±	0		
12	HS	+	+												
	EW			0	0							0	0		
13	EW	+	0												
16	HS					0	0								
17	HS							+	±	+	+++				
	EW					++	0								
18	EW							++	±	0	0				
	HS													+	0
19	EW													0	0
20	HS			0	0							0	0		
21	EW			0	0							±	0		
22	HS	++	+++												
23	EW	+	0												
26	HS					+	0	+	+	++	0				
27	EW					++	0	+++	+	0	0				
28	HS			0	0									++	0
	EW													+	0
29	HS											+	0		
	EW			0	0										
30	EW														
31	HS	+++	0									0	0		
32	EW	0	0												
35	HS					+++	0								
36	HS							±	0	0	0				
	EW					+	0	+	0	0	0				
39	HS			0	0										
	EW			0	0							0	0		
41	HS	0	0									0	0		
	EW	++	0												
45	HS					0	0								
	EW					0	0								

parts adjacent to the sac. It is not unlikely that in just this area a concentration of antigen following intravenous injection occurred which was tantamount to sensitization. A subsequent intravenous injection of antigen could therefore elicit a focal reaction, as indeed it did.

As a further control procedure, 3 dogs (No. 53 and 70, horse serum injected, and No. 58, saline sac without injury) received intravenous injections of egg white following at least two previous intravenous injections of horse serum. None of these dogs reacted to this first contact with a new antigen. Subsequent intravenous injections of horse serum caused general reactions, thereby demonstrating the specificity of the responses observed.

It is also of interest to note that after the second intravenous injection of horse serum No. 70 exhibited a marked reddening of the scalp, accompanied by itching, indicating a sensitization of the skin in this region. This phenomenon was likewise observed in another dog sensitized to egg white (No. 40) included in a previous series of animals.<sup>2</sup>

#### EGG WHITE

Among the dogs receiving egg white in the brain, none exhibited a local reaction following the first intravenous injection of the same antigen. Neither did any of the control, or saline-treated animals. However, No. 68, which had received egg white directly into the brain by injection, showed a moderately severe generalized anaphylactic reaction.

Upon repeated intravenous injections of egg white, all but one of the control dogs responded with general reactions. Our interpretation of this phenomenon is the same as that in the case of the animals reacting to repeated intravenous injections of horse serum. Namely, the first intravenous injection of antigen may act as a sensitizing dose by selective adsorption in traumatized brain tissue. Subsequent intravenous injections therefore may cause a local reaction. The protocol of No. 58 (saline sac without injury) is illustrative. After the first intravenous injection of egg white there was no reaction; after the second intravenous injection there was a moderate general reaction (++); after the third intravenous injection there was a severe general reaction (+++) and a moderate local reaction (++) .

The failure to obtain any reaction in the dogs with egg white sacs following the first intravenous injection of antigen indicates that the antigen may not have come in contact with brain tissue, i.e., that the sac was impermeable to the protein employed. It was very difficult to prepare collodion sacs of such small size (0.3 c.c. capacity) as seemed desirable to use. Furthermore, it should be noted that they were not all tested prior to use as they should have been. The failure to react to intravenous injections by dogs which had received egg white intracerebrally can be accounted for by assuming that there was insufficient contact of antigen with brain tissue to ensure sensitization, i.e., that the antigen was eliminated too rapidly from the local site.

#### HORSE SERUM AND EGG WHITE

Turning now to a consideration of the dogs which received both horse serum and egg white on opposite sides of the brain, we find that one dog (No. 67,

## MONOPHYLETIC SCHEME OF BLOOD CELL FORMATION FOR CLINICAL AND LABORATORY REFERENCE\*

KATSUJI KATO, PH.D., M.D., CHICAGO, ILL.

THE origin, development and interrelationships of the formed elements of the blood have furnished a fertile soil for many controversies among anatomists and pathologists. The average clinician, however, has not given much thought to this aspect of laboratory medicine, simply because of an almost unsurmountable degree of confusion existing among the hematologists. Yet it cannot be denied that a practical knowledge of hemocytogenesis is essential to the clinicians, not only for diagnosis of various types of blood dyscrasias, but for a proper evaluation of other pathologic processes as reflected in the blood. It is, of course, a matter of personal choice as to just which theory a clinician should follow. But it is important that a workable theory be chosen, and in order to use it intelligently for clinical purposes, it must be diligently adhered to.

Since the various theories of blood cell formation have been reviewed from time to time, it is not necessary to repeat the task in detail here. Broadly speaking, all schools of hematologic thought may be classified under either monophyletism or polyphyletism. The monophyletic view includes both the so-called extreme unitarians (Dominici, Weidenreich, Dantschakoff, Maximow, Jolly), and the neounitarians (Pappenheim, Ferrata, Downey). The polyphyletic school consists of the dualists (Ehrlich, Nägeli, Schridde, Piney, and Helly), and the trialists (Schilling, Rosenthal), and the extreme polyphyletists (Sabin and her coworkers). The grounds on which these various theories are based are chiefly of academic interest. But from a practical clinical point of view, the monophyletic theory is by far the most satisfactory.

The scheme of the formation and interrelationships of the blood cells here delineated has been found useful as a ready reference in the laboratory and classroom. Monophyletic in principle, its general form has been suggested by a somewhat similar scheme devised by Epstein and published by Downey. All the blood cells illustrated in the chart have been seen by me either in the peripheral blood or in sections of the hemopoietic organs. Their positions are arranged in developmental sequences, and the following explanatory remarks attempt to redefine and properly classify the existing complexity of hematologic nomenclature.

This scheme is made up of five concentric circular zones, numbered by Arabic numerals 1 to 5, which represent principal stages of maturation of various cell types. Only those stages that are clinically useful in making a satisfactory classification of the cells in the differential count have been illustrated.

\*From the Department of Pediatrics, The University of Chicago.  
Received for publication, November 10, 1934.

intracerebral injections) responded with a marked contralateral focal and slight general reaction to its first intravenous injection of egg white. Another dog (No. 54, saes without injury), after its first intravenous injection of horse serum, exhibited a focal reaction of very slight degree accompanied by a severe general reaction. Control animals, saline-treated, gave no reaction to their first intravenous injection of either antigen. Subsequent intravenous injections of both antigens produced focal and general reactions in all but one dog in this group. An interpretation of this phenomenon has already been suggested.

#### DISCUSSION

The sensitization of brain tissue to horse serum or egg white is beset with serious technical difficulties. Positive evidence of *local* as well as general cerebral anaphylaxis has been presented in detail. Manifest reactions following the *first* intravenous injection of the appropriate antigen in the brain are conclusive. Reactions following repeated intravenous injections of the appropriate antigen in dogs subjected to the brain operations described are indirect substantiation. The results presented here lend further confirmation to those previously reported by us.<sup>1, 2</sup>

The work of Clere, Paris and Sterne<sup>2</sup> seemed to offer a further possibility of obtaining positive responses in animals suitably treated. Consequently, a saturated solution of octanol in 33 per cent ethyl alcohol was injected intravenously (1.7 c.c. per kg. of body weight) two minutes before the last antigen injection. In addition to a marked narcotic effect there were some slight general but no local reactions. When the octanol was injected ten minutes after the antigen, the results were similar.

This investigation is being continued in an effort to perfect a method which will induce local cerebral anaphylaxis in the dog at will.

#### SUMMARY

1. Dogs having normal horse serum or fresh egg white in contact with one side of the brain either by means of collodion sacs or by direct injection, reacted focally *on the opposite side of the body* following an intravenous injection of the appropriate antigen. General reactions were also observed. Control animals failed to respond.

2. A number of dogs subjected to the brain operations described, failed to react to the first intravenous injection of antigen. However, upon repeated injections of the same antigen, contralateral focal (as well as general) reactions were elicited. This phenomenon was interpreted as the result of a selective adsorption of antigen in the traumatized brain tissue causing sensitization.

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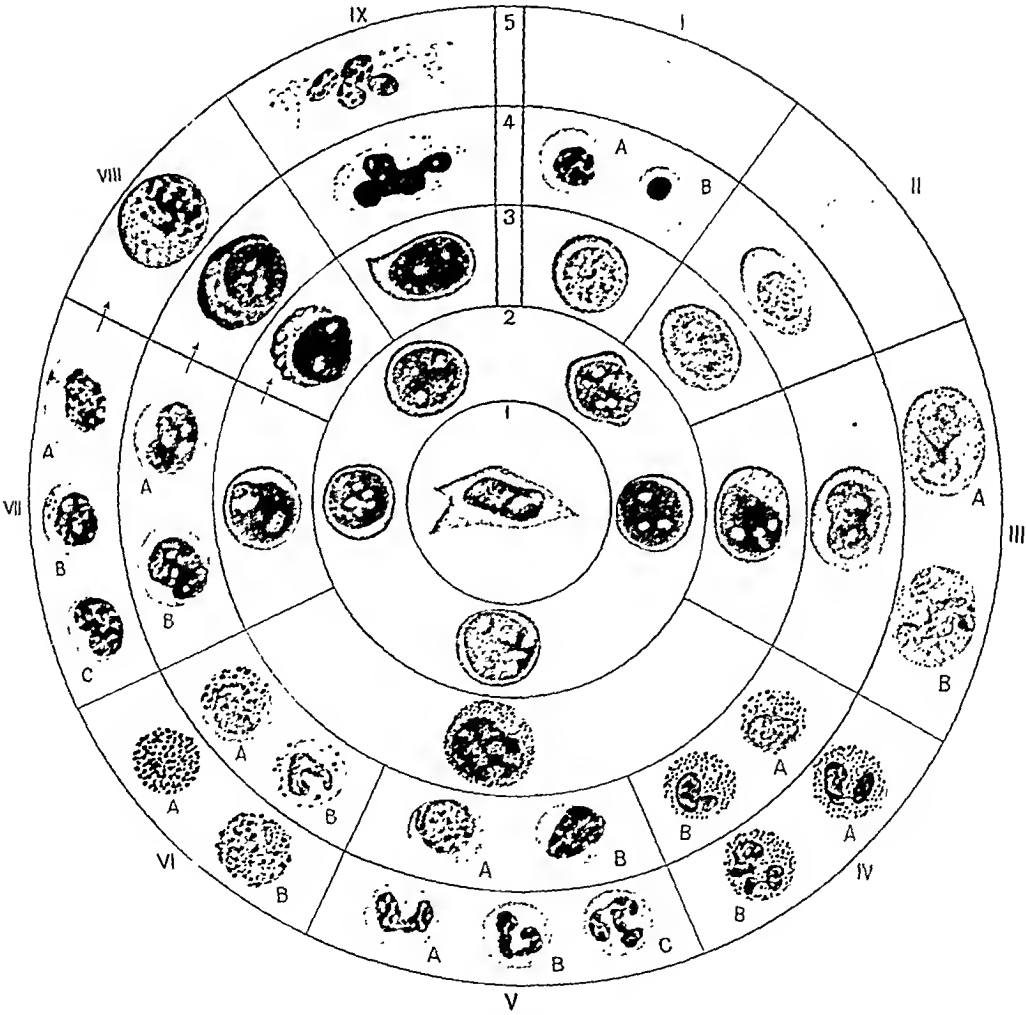


CHART I.



The central circle contains the generally recognized parent cell of all types of the blood cells, while in the outermost zone are grouped all the mature cells found in the peripheral circulation of normal human subjects. The intermediate zones comprise the cells normally found only in the hemopoietic organs, which, under abnormal conditions, may appear in the peripheral blood. Generally speaking, the more pronounced the degree of abnormal stimulation of the hemopoietic tissues, the greater the number and types of the immature cells which appear in the blood stream. This is best exemplified by the greater percentage of the myeloblasts seen in acute myelogenous leucemia in contrast to the chronic stage in which the myeloblasts are comparatively few.

The radiating lines, starting from the second and third zones and extending to the peripheral zone, divide the blood cells into nine distinct and specific groups, numbered clockwise by Roman numerals, I to IX. The fact that these radiating lines do not traverse the first and second zones indicates that the cells in these zones have general relationships to all specific cell groups. This discussion follows the various cell series of each sector through the zone sequences in numerical order. These illustrations are based on the actual appearance of the cells in the blood smears made by the method previously described by me and prepared by the panoptic combination method of Pappenheim (May Grünwald stain, followed by dilute Giemsa solution). It may be stated that the Wright stain, when properly applied, also brings out all the characteristic details here described. The names of all cells illustrated in the scheme are given in the *Genealogy of Hemocytes* accompanying the chart (Table I).

The precursor of all types of blood cells is the undifferentiated mesenchymal cell of the embryo (Zone 1) corresponding in the adult organism to the resting reticulo-endothelial cell, a fixed tissue cell which is not a hemocyte. This cell, however, possesses unlimited mesenchymal developmental potencies, differentiating under normal conditions into the myeloblast, the first general stem cell of the blood. The regeneration of the blood cells beyond the myeloblast stage normally proceeds according to a homoplastic principle. It is only under extremely pathologic conditions that heteroplastic proliferation occurs.

The term myeloblast, according to the unitarian theory here adopted, is applied to the first hemocyte formed by the differentiation of the reticulo-endothelial cell. It is a general hemoblast, a common parent to all the nine specific types of blood cell. In the chart, the five myeloblasts shown in the second zone are all morphologically identical, characterized chiefly by the peculiarly constant pattern of the nuclei. The deeply purple chromatin granules of the myeloblast nucleus are very fine and uniformly distributed. The nucleus itself is relatively large, occupying the major area of the entire cell. There are usually visible nucleoli, varying in number from two to ten. The cytoplasm, scant in amount, usually stains muddy blue, particularly at the periphery. Immediately surrounding the nucleus, there may appear a narrow, slightly creamy, hyaline-clear zone. The bluish-stained cytoplasmic rim often appears granular, especially when seen in company with granulocytes (as in myelogenous leucemia) and erythroblasts (as in erythroblastosis).

Sometimes, however, as seen in lymphatic leucemia, this cytoplasmic ring may appear homogenous. The myeloblast thus described is morphologically identical with the myeloblast of Nägeli, Downey, and Piney. In the literature, it is synonymously known as the lymphoidocyte (Pappenheim), the indifferent lymphoid cell of the marrow (Michaelis and Wolff), and the hemocyto blast (Ferrata). Maximow, however, was unable to distinguish the lymphocyte and the myeloblast on the basis of their cytologic structures, and his hemocyto blast is not the same cell as the myeloblast here described.

Sectors I and II depict the cells of the erythrocytic series. It is now generally agreed that the cells of the normoblastic series represent the definitive or permanent erythrocytes in the normal adult organism, replacing the primitive or embryonic erythrocytes of the megaloblastic group. These two series of erythrocytes, therefore, are distinct not only in their cytologic structures,

TABLE I  
GENEALOGY OF HEMOCYTES

ZONE 1 PARENT CELL	ZONE 2 GENERAL HEMO- BLAST	ZONE 3 SPECIFIC HEMOBLASTS	ZONE 4 INTERMEDIATE HEMOCYTES	ZONE 5 MATURE HEMOCYTES OF CIRCULATION	SECTOR
Undifferentiated mesenchymal cell (embryo)  or  Reticuloendo- thelial cell (adult)	Myelo- blast	Pronormoblast (Proerythro- blast)	A. Polychromatic normoblast B. Orthochromatic normoblast	Orthochromatic erythrocyte	Sector I
		Promegaloblast	Megaloblast	Megalocyte	Sector II
		Monoblast	Promonocyte	A - B Monocytes	Sector III
		Leucoblast	A. Eosinophilic myelocyte B. Eosinophilic metamyelocyte	A - B Eosinophilic poly- morphonuclears	Sector IV
			A. Neutrophilic promyelocyte B. Neutrophilic myelocyte	A. Neutrophilic metamyelocyte B. Neutrophilic staff cell C. Neutrophilic polymorpho- nuclears	Sector V
			A. Basophilic myelocyte B. Basophilic meta- myelocyte	A - B Basophilic poly- morphonuclears	Sector VI
		Lymphoblast	A - B Prolymphocytes	A. Large lympho- cyte B. Medium lympho- cyte C. Small lymphocyte	Sector VII
		Plasmoblast (Lympho- blastic plasma cell)	Proplasmocyte	Plasmocyte (Plasma cell)	Sector VIII
		Megakaryo- blast	Megakaryocyte	Thromboocytes (platelets) and nuclear frag- ments of mega- karyocyte	Sector IX



the cell. The final mature cell of the megaloblastic group is the megalocyte (Sector II, Zone 5) which, after having passed the various stages of nuclear condensation, has completely cast out its nucleus.

The normal formation and regeneration of the red blood cells in the adult organism involve only the normoblastic series, but under pathologic conditions the megaloblastic group may be stimulated. Clinically there are only a very few conditions in which such stimulation is reflected in the blood stream, chief among which are pernicious anemia, intestinal parasites, calcinomatosis and severe types of erythroblastosis in infants. In fetal hemopoiesis, the megaloblastic form of regeneration is said to be a normal function and such a phenomenon may be found occasionally and only temporarily in postnatal life of prematurely born infants.

Sector III contains the cells of the monoeytic series, of which the monoblast (Zone 3) is the most primitive. The monoblast is characterized by a slight tendency of the chromatin granules to become reticular. The nucleoli are definitely visible. The granular cytoplasm is still deeply basophilic, though usually not as marked as that of the proerythroblast or the promegaloblast. The cell in the fourth zone of Sector III is the promonocyte, characterized by a still further condensation of reticular chromatin strands, with a frequently visible nucleolus. Its cytoplasm is basophilic, less pronounced than that of the monoblast, and shows a greater degree of granular appearance. Fine azur granules are only occasionally seen, being frequently absent altogether. This type of cell is occasionally seen in the peripheral circulation under conditions of excessive stimulation of the reticulo-endothelial system. The mature monocytes of the peripheral blood (Sector III, Zone 5, A and B) are characterized by the pale bluish granules in the cytoplasm and an abundance of nonspecific fine azur granules, often spoken of as azur dust. Their nuclear pattern is highly reticular in its chromatin arrangement. The configurations of the nuclei are greatly varied, sometimes showing a kidney-beanlike indentation on one side, in the deep crevice of which upon close examination are usually seen two small projections; or again the nuclei may be greatly elongated, folding and bending their extended nuclear processes upon one another, producing fantastic shapes of infinite varieties. This latter type of monoeyte is sometimes referred to as a Rieder cell.

Sectors IV, V, and VI illustrate the three recognized varieties of specific granulocytes, of which the leucoblast (Pappenheim) is the common precursor, the first distinct and specific stem cell differentiating in the granulocytic direction from the myeloblast, its general stem cell. The leucoblast (Sectors IV, V, VI, Zone 3) is characterized by its large size and slightly reticular nucleus with distinctly visible nucleoli. Its nuclear membrane is usually invisible and cytoplasm moderately blue in staining though lighter than the blue of the myeloblast. The most distinguishing feature, however, of the leucoblast is the abundance of nonspecific azur granules, scattered profusely over the surface of the entire cell, ranging in number from about twenty to sometimes one hundred or more. It is from this common specific hemoblast

but also in their functional significance. In the clinical literature, the megaloblast is frequently referred to as representing the most immature stage in the development of the normoblastic erythrocytes, but such a view is the result of confusing the phylogenetic position of the megaloblast with the ontogeny of the normoblast.

In the normocytic series, the most primitive cell is the proerythroblast (Sector I, Zone 3), a cell referred to in the literature also as the basophile erythroblast (Ferrata) or the macroblast (Naegeli). This cell corresponds to the promegaloblast in the degree of maturity, their chief difference being their characteristic nuclear pattern. The chromatin granules in the pronormoblast, show a greater tendency to aggregation than in the promegaloblast, clumping of chromatin in the former cell already suggesting a cart wheel arrangement. The cytoplasm, deeply blue and somewhat granular, appears much more condensed and irregular in outline than in the promegaloblast. The presence of hemoglobin is not yet detectable.

The early normoblast (Sector I, Zone 4, A) being a more mature nucleated red blood cell, definitely demonstrates the presence of hemoglobin. The nuclear chromatin is characteristically arranged in cart wheel shape, though the cytoplasm may be still polychromatophilic. The nuclear membrane is usually heavy and distinctly visible. In a later stage, this polychromasia of the cytoplasm is almost completely replaced by pure hemoglobin, but with a greatly condensed nucleus (Sector I, Zone 4, B). The cell is then called orthochromatic normoblast. This latter nucleus is finally lost by extrusion, with the resulting mature erythrocyte (Sector I, Zone 5). Such terms as the orthocnormocyte, the macronormocyte, and the micronormocyte, used by some hematologists, are all mature erythrocytes of this series, differentiated on the basis of their cytologic dimensions.

The cells of the megaloblastic group are illustrated in Sector II. The first distinct cell of this series, called promegaloblast (Sector II, Zone 3), is characterized by somewhat coarse chromatin granules, uniformly distributed throughout the nucleus, and with very little tendency to form into aggregations. The nuclear membrane is indistinct and the nucleolus sometimes visible. The parachromatin spaces are much more conspicuous than in the stem cell, and the cytoplasm, still very dark blue and rather granular. As a rule, this promegaloblast is much larger in dimensions than the comparable cell of the normoblast series, the pronormoblast. Like the latter cell, the promegaloblast shows no evidence of hemoglobin in its protoplasm.

The megaloblast is shown in the fourth zone of Sector II. This cell is characterized by its huge size, and by its primitive nuclear pattern, which, however, is beginning to show some evidence of chromatin condensation, causing the nucleus to appear coarsely reticular. The nuclear membrane is usually very indistinct. The most characteristic aspect of this cell is the fairly abundant presence of hemoglobin, in sharp contrast to the cells of similar nuclear immaturity in the normoblastic series. The slightly polychromatophilic cytoplasm usually presents granular appearance, especially near the periphery of

the cell. The final mature cell of the megaloblastic group is the megalocyte (Sector II, Zone 5) which, after having passed the various stages of nuclear condensation, has completely cast out its nucleus.

The normal formation and regeneration of the red blood cells in the adult organism involve only the normoblastic series, but under pathologic conditions the megaloblastic group may be stimulated. Clinically there are only a very few conditions in which such stimulation is reflected in the blood stream, chief among which are pernicious anemia, intestinal parasites, calcinomatosis and severe types of erythroblastosis in infants. In fetal hemopoiesis, the megaloblastic form of regeneration is said to be a normal function and such a phenomenon may be found occasionally and only temporarily in postnatal life of prematurely born infants.

Sector III contains the cells of the monocytic series, of which the monoblast (Zone 3) is the most primitive. The monoblast is characterized by a slight tendency of the chromatin granules to become reticular. The nucleoli are definitely visible. The granular cytoplasm is still deeply basophilic, though usually not as marked as that of the proerythroblast or the promegaloblast. The cell in the fourth zone of Sector III is the promonocyte, characterized by a still further condensation of reticular chromatin strands, with a frequently visible nucleolus. Its cytoplasm is basophilic, less pronounced than that of the monoblast, and shows a greater degree of granular appearance. Fine azur granules are only occasionally seen, being frequently absent altogether. This type of cell is occasionally seen in the peripheral circulation under conditions of excessive stimulation of the reticulo-endothelial system. The mature monocytes of the peripheral blood (Sector III, Zone 5, A and B) are characterized by the pale bluish granules in the cytoplasm and an abundance of nonspecific fine azur granules, often spoken of as azur dust. Their nuclear pattern is highly reticular in its chromatin arrangement. The configurations of the nuclei are greatly varied, sometimes showing a kidney-beanlike indentation on one side, in the deep crevice of which upon close examination are usually seen two small projections; or again the nuclei may be greatly elongated, folding and bending their extended nuclear processes upon one another, producing fantastic shapes of infinite varieties. This latter type of monocyte is sometimes referred to as a Rieder cell.

Sectors IV, V, and VI illustrate the three recognized varieties of specific granulocytes, of which the leucoblast (Pappenheim) is the common precursor, the first distinct and specific stem cell differentiating in the granulocytic direction from the myeloblast, its general stem cell. The leucoblast (Sectors IV, V, VI, Zone 3) is characterized by its large size and slightly reticular nucleus with distinctly visible nucleoli. Its nuclear membrane is usually invisible and cytoplasm moderately blue in staining though lighter than the blue of the myeloblast. The most distinguishing feature, however, of the leucoblast is the abundance of nonspecific azur granules, scattered profusely over the surface of the entire cell, ranging in number from about twenty to sometimes one hundred or more. It is from this common specific hemoblast

that arise the specifically granulated promyelocytes, myelocytes, metamyelocytes, and finally mature polymorphonuclear leucocytes.

In Sector IV, Zone 4, are shown the eosinophilic myelocyte (*A*), and metamyelocyte (*B*), which develop into mature eosinophilic granulocytes with segmented nuclei (Sector IV, Zone 5, *A* and *B*). In the eosinophilic myelocyte, the specific granules do not always appear typically red, but rather dark grayish, due to the immature state of some granules. The nucleus of the mature eosinophilic granulocyte usually is made up of two elongated segments, resembling a pair of butterfly wings, but in pathologic conditions, it shows a greater number of segments.

The neutrophilic promyelocyte (Sector V, Zone 3, *A*), characterized by the coexistence of both the primitive basophilic cytoplasm and the specific neutrophilic granules, is the precursor of the neutrophilic myelocyte. In the latter cell (Sector V, Zone 3, *B*) the entire cytoplasm is converted into mature specific neutrophilic granules. The nuclei of both cells are still undifferentiated, but the chromatin aggregation is pronounced.

Sector V, Zone 5, *A*, *B*, and *C* illustrates the three degrees of mature neutrophilic granulocytes in normal circulation, respectively designated as the metamyelocyte, the staff neutrophile and the segmented neutrophile, in order of maturity. The youngest of these, the metamyelocytes, is found normally only in the blood of the newborn infants during the first few days of postnatal life. The staff cells characterized by a further differentiation of the nucleus, but not yet showing its segmentation, are also normally present in the peripheral blood, their increase indicating a correspondingly good prognosis in cases of acute infection. The typical segmentation of the nucleus, as in mature granulocytes, must always show the presence of two or more segments of the nuclear substance, mutually connected by a very thin filament of the same substance. Occasionally nuclear buds may be seen projecting from any portion of the nuclear lobules. As the granulocyte matures, the number of segments increases, and in certain stages as many as ten or twelve lobules may be distinguished. The so-called qualitative differential count of the Arneeth hemogram is based on the number of segments thus demonstrated. Occasionally one or two light bluish protoplasmic granules of varying sizes may be found in the cytoplasm of segmented neutrophilic granulocytes. They have been called Döhle's inclusion bodies, but seem to possess no clinical significance.

The specific basophilic myelocyte (Sector VI, Zone 4, *A*) possesses a rather grayish blue cytoplasm with a rounded configuration of the nucleus containing characteristically clumped chromatin granules. The morphology of the basophilic metamyelocyte (Sector VI, Zone 4, *B*) is exactly identical with that of the myelocyte, except the presence of one deep indentation of the nucleus on one side. The specific granules may be either scant or abundant, though never as plentiful as the granules of the mature basophilic granulocyte, the nucleus of which is segmented (Sector VI, Zone 5, *A* and *B*). The basophilic granulocyte is synonymously known as the mast cell in the older literature.

The cells of the lymphocytic series are shown in Sector VII, the most primitive being the lymphoblast (Zone 3) which develops from the myeloblast

as may be traced in the blood of subacute and acute lymphatic leucemia. This lymphoblast is the specific stem cell for all types of lymphocytes. In general structure the cell is definitely lymphocytic, in that the chromatin granules are much coarser than in its parent cell, the myeloblast. The cytoplasm becomes a little more abundant as compared with the myeloblast, and the nucleoli, usually two in number, are distinctly visible. The more mature cells of this series are the large and small prolymphocytes, morphologically identical (Sector VII, Zone 4, *A* and *B*). Their nuclei contain definitely characteristic chromatin aggregations with occasionally visible single nucleoli. Their cytoplasm, peculiarly hyaline, stains sky-blue with no protoplasmic granulations. From either these large or these small prolymphocytes, the three recognized varieties of mature lymphocytes develop, but the exact antecedent of each has not been clearly established. The large lymphocyte (Sector VII, Zone 5, *A*) has characteristic aggregation of chromatin into heavily staining bars and clumps. The cytoplasm, hyaline, transparent and homogeneous, often displays a tendency toward irregular protoplasmic distribution of blue staining. Occupying an intermediate position among the mature lymphocytes, the medium lymphocyte or mesolymphocyte (Sector VII, Zone 5, *B*) is identical with the large type in morphologic details, except in the occasional presence of visible non-specific azur granules much coarser than those seen in the monocytes. These granules are practically never seen in abundance and occur at one portion of the hyaline cytoplasm usually near the nuclear indentation. The small lymphocyte (Sector VII, Zone 5, *C*), characterized by a very small amount of cytoplasm, is frequently more definitely condensed as to nuclear chromatin than the two lymphocytes above described.

The lymphocytes have chiefly been the center of many hematologic controversies, because of their developmental potencies exhibited under pathologic conditions. Some unitarians believe that the mature lymphocytes of the normal blood, particularly the small variety, may dedifferentiate under abnormal stimulation back into the myeloblast, through the lymphoblast stage. This myeloblast may in turn redifferentiate into any type of hemocyte. Thus the blood picture reported by Logeheil under the title of mixed leucemia may be interpreted to mean only a certain stage of myeloid leucemia, in which the lymphocytes are dedifferentiating back into the myeloblast and then finally into the granulocytes.

In Sector VIII are shown three varieties of hemocytes usually called plasma cells (Cajal, Unna, Marschalko, Schridde). The typical plasma cells are present in the normal blood in a small percentage and, therefore, their occurrence has no pathologic implications. The usual type, here called plasmocyte (Zone 5), is characterized by its ultramarine blue, opaque cytoplasm, somewhat granular, a nucleus often eccentrically placed, and a chromatin aggregation usually in cart wheel arrangement. Also, just adjacent to the nucleus, appears a narrow clear zone sharply contrasting with the opaquely blue cytoplasm. The plasma cell with a typical cytoplasm but with an immature nuclear pattern has been recognized by some observers to belong to the plasmocytic series. Such a cell has been called lymphoblastic plasma cell



(Schridde), and it is derived directly from the lymphoblast. In the present scheme, this variety of plasma cell is placed in Sector VIII, Zone 3, and tentatively called plasmoblast. It is conceivable that this apparently primitive cell may give rise to a more typical plasma cell. The so-called irritation form of Türk, according to some investigators, may be identical with the lymphoblastic plasma cell, as its nuclear pattern is found to be immature. When it occurs in myelogenous leucemia, it is undoubtedly derived from the myeloblast. Again, a cell which appears to possess a nuclear chromatin arrangement of intermediate maturity, here called proplasmocyte (Sector VIII, Zone 4), may be found in the organs of such conditions as plasma cell leucemia (lympho-adenosis leucemica plasmacellularis, Piney) and plasmaeytoma or plasma cell myeloma. Occasionally, the plasma cell may show, in its cytoplasm, the presence of nonspecific azur granules (plasmacytoid cell of Piney). Vacuoles are also frequently noted in the cytoplasm of all types of plasma cells.

The cells of the plasmocytic group offer the greatest difficulty in establishing their respective positions in the developmental scale. The genetic arrangement here proposed is merely an attempt to classify the cells of this group solely on the basis of chromatin immaturity. It does not, therefore, necessarily represent the cytogenetic sequence. The histologic evidence at present available points very strongly to the lymphocytic origin of most of the plasmocytes (Marschalko, Krompecher, Naegeli, Jolly, Maximow, Downey), although there are a number of investigators who hold a histogenous origin from connective tissue cells such as elasmatoocytes, resting wandering cells, and adventitial cells (Unna, Cajal, Pappenheim, Marchand, Foa, Türk). Again, some observers believe that the plasma cells originate from immature blood cells, such as myeloblasts and erythroblasts through aberration and abortion (McGowan, Jordan, Dawson and Masur). While most plasma cells seem to be derived from mature lymphocytes, there is much evidence to indicate that all of the cell types enumerated above may transform to plasma cells which, however, do not necessarily acquire the cart wheel nucleus. This indicates that plasma cell formation is a reaction which may involve several types of cells, more or less closely related among themselves (Downey), and may include even the fibroblasts (Unna).

The various stages in the development of the megakaryocyte and platelets are traced in Sector IX. Most primitive is the megakaryoblast (Sector IX, Zone 3). Surrounded by a definitely hyaline zone and containing distinctly visible nucleoli, its nucleus is marked by a somewhat densely arranged reticular chromatin pattern. The cytoplasm is slate gray. This cell develops into the typical megakaryocyte (Sector IX, Zone 4), a mature cell of huge dimensions, with a characteristic multilobulated large nucleus, taking an extremely deep stain. The cytoplasm, hyaline in ground substance, contains numerous fine purplish-stained granules. Portions of the cytoplasm may be seen breaking off as individual platelets, here called thrombocytes. Normally the megakaryocyte is found in the bone marrow of adult subjects and also in the hepatic sinusoids of embryonic organs. The typical thrombocyte consists of two distinct parts: the chromomere which is highly refractile and composed

of purple granules, and the hyalomere, taking on a pale blue stain, homogeneous in appearance and extremely flexible. The individual platelets vary greatly in size, the average being 3 micra in diameter. They tend to agglutinate with one another, forming irregular islands on the smear. Frequently large masses, possessing every property of a typical platelet, are found. The thrombocytes are normal constituents of the peripheral blood, and occasionally even fragments of megakaryocytic nucleus may be found under normal conditions. Pathologically the platelets may be greatly reduced (as in thrombocytopenic purpura) or much increased (as in posttraumatic hemorrhage and myelogenous leucemia).

It is, in conclusion, to be emphasized that the foregoing conception is not an attempt to formulate any new theory of hemoctyogenesis. Rather it has endeavored only to: (1) systematize the hematologic nomenclature; (2) to redefine the various cell types (chosen wholly on the basis of clinical significance); and (3) to arrange them in proper genetic relationship according to the monophyletic (neounitarian) theory.

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## SOME OBSERVATIONS ON THE COMPARATIVE EFFECTIVENESS OF MERCURIAL DIURETICS WITH AND WITHOUT THEOPHYLLINE (MERCUPURIN, SALYRGAN, ETC.)\*

MARSHALL N. FULTON, M.D., BOSTON, MASS., AND A. HUGHES BRYAN, M.D.,  
CHICAGO, ILL.

**B**ECAUSE of their marked diuretic effect, generally superior to that of xanthine compounds, the mercurial diuretics now occupy an important place in the treatment of patients with edema. The purpose of this communication is to report our early experiences with two new mercurial diuretics. (1) mercupurin, which contains theophylline both free and chemically bound with a complex organic mercury compound, structurally quite different from salyrgan, and (2) a combination of a mercurial diuretic to which free theophylline has been added in a concentration of 5 per cent. The latter combination, which is not available commercially, will be referred to in this paper as diuretic X.

The first of the mercurial diuretics to attain common use, merbaphen or novasurol, was found to cause toxic manifestations in patients. Though excellent in its diuretic effect, its continued use frequently led to such symptoms as salivation, gingivitis, colitis, and proctitis.<sup>1</sup> Johnstone and Keith<sup>2</sup> found that in rabbits it produced renal lesions identical with those caused by mercurial compounds in common use, although its toxicity appeared to be less than that of mercuric chloride when based on the amount of pure mercury contained in the two.

Mersalyl or salyrgan was introduced in this country about 1927. Although containing a slightly higher percentage of mercury than novasurol (39 per cent compared with 33 per cent), it was found to be much less toxic to experimental animals and to man, and to have as satisfactory a diuretic effect, if indeed not a better one, than novasurol. It has been suggested<sup>3, 4</sup> that the difference in toxicity of the various mercurial compounds depends on

\*From the Medical Clinic, Peter Bent Brigham Hospital, and The Department of Medicine, Harvard Medical School.

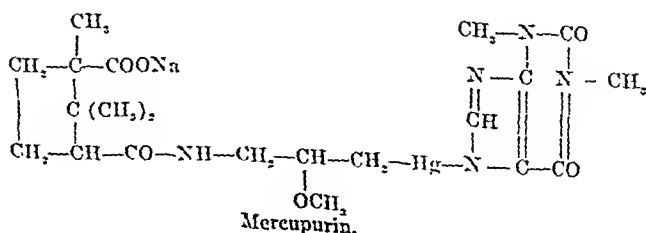
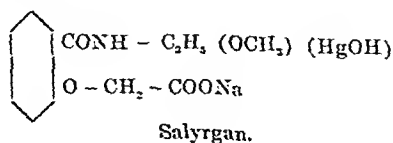
Received for publication, March 1, 1935.

the chemical linkage of the mercury with the rest of the molecule rather than the actual percentage present. Though isolated reports of ill effects attributed to salyrgan have appeared in the literature,<sup>5, 6</sup> the absence of toxic effects following its use has been noted repeatedly. Several reports have appeared<sup>7, 8</sup> of patients to whom salyrgan has been administered over a period of five years at six- to seven-day intervals without causing any untoward symptoms or without diminution of its effectiveness. Tarr and Jacobson<sup>1</sup> encountered only two instances of mild stomatitis in a group of patients receiving more than 3,000 injections of this drug. In thirty autopsies on patients who had been given from 3 to 130 c.c. of salyrgan, they found only one instance in which the kidneys showed a lesion suggestive of injury from mercury. In this laboratory a daily injection for thirty days of 1 c.c. of salyrgan intravenously into a dog weighing eighteen kilos produced no untoward symptoms, and the kidneys at autopsy showed no abnormality microscopically. The chief disadvantages to the use of salyrgan are (1) its ineffectiveness by the oral route, (2) its tendency to cause venous thrombosis and occasionally necrotic skin lesions at the site of injection, and (3) the pain which it causes on intramuscular injection. However, because of its excellent diuretic effect and the absence of significant toxic sequelae following its repeated administration, salyrgan has become at present a most widely used and popular diuretic drug.

Is a more ideal mercurial diuretic available?

A number of complex organic mercury salts have been tried out from time to time in the hope of securing, if possible, other compounds of low toxicity which have a pronounced diuretic effect. In 1928 v. Issekutz and v. Vegh<sup>9</sup> reported on the comparative value of a group of mercurial compounds, studying the diuretic action of these substances in rabbits and their toxicity in rats. They found that their preparation No. VI, when combined with theophylline, yielded the best results. In this form it increased the urine flow in rabbits considerably more than either salyrgan or novasurol and was relatively non-toxic.

A combination similar to, if not identical with, this last drug has been in use for several years in European clinics under the name of novurit and has been introduced recently in this country as mercupurin. Chemically this substance is distinctly different from salyrgan as shown by the formulas:



The mercurial salt of mercupurin contains 41 per cent mercury. An important difference between salyrgan and mercupurin is that the mercurial salt of the latter has bound with it chemically 3.5 per cent of theophylline. To this is added 1.5 per cent of free theophylline. Reports in the European literature vary as to the effectiveness of this drug in comparison with salyrgan and novasurol. Thus, some authors have found it at times more effective,<sup>10, 11</sup> while others have reported no marked difference.<sup>12, 3</sup> It has been given repeatedly both intravenously and intramuscularly without causing toxic effects. Hahn<sup>3</sup> reported freedom from pain in patients following intramuscular injection of novurit (mercupurin) and a greater diuresis than with salyrgan during the first four hours. The twenty-four-hour output, however, was not essentially different with the two drugs. Crawford<sup>12</sup> has studied recently the comparative effectiveness of salyrgan and mercupurin in a group of edematous patients, finding mercupurin the more efficacious of the two in the majority of cases, though not in all.

DeGraff and others<sup>16</sup> found in cardiac patients that the diuretic effect of mercupurin is greater than that of mercurial diuretics not containing theophylline. They attribute this superiority chiefly to the presence of theophylline.

The other preparation used in this study, diuretic X, was a recognized mercurial diuretic to which was added theophylline in the strength of 5 per cent, an amount comparable to that contained in mercupurin.

Certain considerations favor the addition of a xanthine compound to a mercurial diuretic as in mercupurin and diuretic X. In the first place the contention is advanced by some that these two types of drugs have different modes of action, the mercurial diuretic affecting chiefly the storage of water in the tissues and the other acting directly on the kidneys. Second, investigators, who believe that both types affect the kidneys predominantly, have presented evidence suggesting that salyrgan acts by decreasing tubular reabsorption, whereas theophylline causes diuresis by increasing glomerular filtration. If either of these arguments is valid, a combination of the two drugs might yield a preparation of considerable diuretic potency. Thus Herrmann and others<sup>13</sup> have advocated the administration of salyrgan along with various xanthine diuretics to obtain a maximum urine output in patients with cardiac edema. Last, v. Issekutz and v. Vegh<sup>6</sup> felt that such a preparation should be less painful to patients when injected intramuscularly than solutions of the mercurial compounds alone, because the weakly acidic properties of theophylline would neutralize the alkaline reaction resulting from the hydrolysis of the complex mercurial salt.

During the past year we have observed the action of these two theophylline-containing diuretic mixtures, comparing them with salyrgan in hospitalized patients and experimental animals. We have been particularly interested in their diuretic potency, in the pain which they occasion when injected intramuscularly into patients, and in the skin reaction following their injection subcutaneously into animals. The results of this study are presented below.\*

\*We wish to express our thanks to the Campbell Products, Inc., 79 Madison Avenue, New York, N. Y., for the mercupurin and to the Winthrop Chemical Co., Inc., 170 Varick Street, New York, N. Y., for the salyrgan used in these studies.

## OBSERVATIONS IN PATIENTS

1. *Mercupurin and Salyrgan*.—The plan of observation with patients, all of whom had edema due to heart failure, was to alternate the diuretics at intervals of three to five days. The subjects were observed first for several days for any evidence of spontaneous diuresis or of that due to digitalis before the diuretics were given. All of them received digitalis as their clinical condition indicated during their stay in the hospital, and the majority received 1.0 gm. of ammonium chloride three to four times daily. Not all patients of this type are entirely satisfactory for determining the comparative effectiveness of different diuretics. The most satisfactory measurements are to be had in those with marked or chronic edema who require not two or three but a dozen good diureses before the edema disappears. Even if a carefully controlled régime with measured and evenly distributed intake of food and fluid is carried out, the extent of the edema will influence considerably the response to a diuretic. Frequently the patient may have a brisk diuresis the first time a diuretic is given and will lose sufficient edema so that the same dose of the same drug given several days later will not induce a comparable diuresis. Last, to avoid presenting a mass of data we have tabulated the average urine output for a given dose of a drug when that drug was given more than once to a patient. Such a method of tabulation is open to criticism, for in instances where a marked diuresis is followed by a relatively slight one, the average might be conspicuously lowered by the second result. All of these facts must be considered in evaluating the data at hand.

The results are presented in Table I. It will be noticed that there are specific instances in which mereupurin caused a significantly greater urine output than salyrgan, while in others the opposite was the case. The general impression created during the time these observations were made was that the two preparations were about equally efficacious in causing diuresis. Neither one produced any definite signs of kidney damage, as judged by the urine, by phthalein excretion or by nitrogen retention, nor did either one lead to other signs of mercurial intoxication. The few times that mereupurin was given intramuscularly it caused pain which seemed to be of no less degree, though shorter in duration, than that usually caused by salyrgan. The diuretic response to intramuscular injection was adequate though somewhat less than that following administration by the intravenous route.

2. *Diuretic X and Salyrgan*.—In the study of diuretic X in this clinic more attention was paid to determining the amount of pain it caused when injected intramuscularly than to a comparison of its diuretic action with that of salyrgan. Inasmuch as this preparation did not cause a slough in dogs and rabbits, when injected intra- or subcutaneously (see below), it was thought that intramuscular injection might be a painless procedure. This was found not to be the case. The patients complained on the whole about as much from the discomfort following diuretic X as from that following salyrgan intramuscularly. In the patients in whom the diuretic response of the two drugs could be compared, no greater diuresis was evident from the presence of theophyl-

line in diuretic X (Table II). It was our impression that the responses to diuretic X were in no way superior to those obtained with salyrgan.

## OBSERVATIONS IN ANIMALS

The comparative action of the three diuretics was measured in normal dogs and rabbits by administering these preparations to the animals at intervals

TABLE I

AVERAGE DIURESIS FOLLOWING MERCUPURIN AND SALYRGAN IN SIXTEEN PATIENTS WITH EDEMA OF CARDIAC FAILURE

MERCUPURIN				SALYRGAN			
PATIENT	NO. OF OBSERVATIONS	AVER. 24-HOUR		NO. OF OBSERVATIONS	AVER. 24-HOUR		DOSE INTRAVENOUSLY
		FLUID INTAKE C.C.	URINE OUTPUT C.C.		FLUID INTAKE C.C.	URINE OUTPUT C.C.	
1	5	710	3,915	4	790	3,215	1 c.c.
2	2	1,150	2,600	3	1,100	3,250	1 c.c.
	3	1,000	5,220	3	950	2,900	1.8 c.c.
3	4	1,175	6,935	4	1,165	6,155	1 c.c.
	3	1,000	6,590	4	1,000	4,750	2 c.c.
4	3	1,170	4,530	4	1,080	4,450	1 c.c.
5	2	1,200	2,350	4	1,150	2,680	1 c.c.
6	1	-	4,000	2	-	3,000	1 c.c.
	2	-	4,625	3	-	4,660	2 c.c.
7	1	1,500	3,575	2	1,100	3,300	1 c.c.
	1	1,000	5,400	1	1,050	3,250	2 c.c.
8	1	1,900	7,110	3	1,500	3,130	1 c.c.
9	1	2,000	5,300	2	1,700	4,475	1 c.c.
10	3	1,060	2,140	2	1,150	2,600	1 c.c.
	1	1,200	3,500	2	1,250	1,950	2 c.c.
11	1	1,000	1,800	1*	900	2,720	1 c.c.
12	1	1,600	4,400	1*	1,000	4,770	1 c.c.
13	1	700	4,400	1*	1,100	4,950	1 c.c.
14	1	1,000	2,400	1*	1,200	1,400	1 c.c.
15	1*	1,500	2,450	1	1,450	1,550	1 c.c.
16	1	1,250	3,950	1*	1,500	3,300	1 c.c.

\*Indicates drug given first.

TABLE II

AVERAGE DIURESIS FOLLOWING DIURETIC X AND SALYRGAN IN SIX PATIENTS WITH EDEMA OF CARDIAC FAILURE

DIURETIC X				SALYRGAN			
PATIENT	NO. OF OBSERVATIONS	AVER. 24-HOUR		NO. OF OBSERVATIONS	AVER. 24-HOUR		DOSE
		FLUID INTAKE C.C.	URINE OUTPUT C.C.		FLUID INTAKE C.C.	URINE OUTPUT C.C.	
1	1*	750	1,950	1	700	2,000	1 c.c. i.m.
2	1	1,300	1,400	1*	1,800	3,000	1 c.c. i.v.
3	1*	1,200	2,700	1	1,200	2,400	2 c.c. i.m.
4	1*	1,100	3,500	2	1,350	1,950	2 c.c. i.m.
5	1	450	1,300	2*	1,100	2,300	2 c.c. i.m.
6	1*	1,100	1,300	1	600	1,750	1 c.c. i.m.

\*Indicates drug given first.

of three to seven days. The animals were kept on a constant diet and were allowed water *ad libitum* until two hours before the diuretic was given. Food was withheld on the day that the measurements were made. The urine was collected in metabolism cages during a six-hour period. The diuretic was given at

the end of the second hour so that its effect was observed for four hours, at the end of which time the bladder was emptied by catheter. As a control, the same procedure was carried out on other days without administering a diuretic. This method affords a satisfactory, though somewhat rough, basis for comparing the effects of diuretics when given repeatedly to the same animals. The urine was measured as to volume and chloride content. To obviate chloride depletion, which will occur from repeated drug-induced diureses, an amount of sodium chloride containing slightly more chloride than that excreted in the urine was administered by stomach tube following the six-hour period of urine collection.

The results are shown in Table III. It was found in rabbits that mercuripurin increased the urine volume 3 to  $3\frac{1}{2}$  times that observed in control

TABLE III

URINE AND CHLORIDE OUTPUT IN RABBITS AND DOGS FOR A SIX-HOUR PERIOD DURING FASTING STATE

NO. OF EXPERIMENTS	NO. OF ANIMALS	AVERAGE URINE VOLUME C.C.	AVERAGE URINE CHLORIDE MG.	
<i>Rabbits</i>				
48	14	18	15.6	Controls
13	9	35	127.0	Salyrgan 0.2 c.c. i.v.
8	4	24	54.8	Salyrgan 0.2 c.c. i.m.
11	8	50	208.0	Mercuripurin 0.2 c.c. i.v.
8	4	65	262.0	Mercuripurin 0.2 c.c. i.m.
11	8	83	216.0	Diuretic X 0.2 c.c. i.v.
7	5	84	301.0	Diuretic X 0.2 c.c. sub. cut.
7	5	73	284.0	Salyrgan 0.2 c.c. i.v. plus Theophylline 10 mg. sub. cut.
<i>Dogs</i>				
7	2	64	35.4	Controls
7	2	143	933.0	Salyrgan 0.75 to 0.8 c.c. i.v.
7	2	188	1,656.0	Mercuripurin 0.75 to 0.8 c.c. i.v.
9	3	199	1,723.0	Diuretic X 0.75 to 0.8 c.c. i.v.

experiments, and the chloride output 13 to 17 times. Salyrgan was less effective in rabbits, augmenting the urine output only from 18 c.c. to 24 c.c. (when given intramuscularly) and to 35 c.c. (when given intravenously). This difference in the response of rabbits to the two drugs may be due largely, if not entirely, to the presence of theophylline in the mercuripurin. Rabbits respond to theophylline with a good diuresis.<sup>14</sup> In fact the largest diuresis observed in these animals occurred with the combination containing free theophylline in the form of diuretic X. With this preparation the urine volume exceeded the control by four to four and one-half times. This occurred following both the intravenous and subcutaneous administration of the drug. Furthermore, when 10 mg. of theophylline (the amount contained in 0.2 c.c. of mercuripurin or diuretic X) were given subcutaneously along with 0.2 c.c. of salyrgan intravenously, the resulting diuresis was definitely greater than that following either mercuripurin or salyrgan alone. These findings point favorably to the



effectiveness of combining the mercurial diuretics with theophylline. However, the fact that diuretic X did not prove to be superior to salyrgan or mercupurin in human beings emphasizes the inadvisability of judging the value of a drug of this type in human beings solely from its action in rabbits.

In dogs the response to the three preparations is shown in Table III. Here, again, the mercurial diuretics in combination with theophylline produced a greater output of urine and chloride than did salyrgan alone. The urine excretion was three times, the chloride output nearly fifty times that observed in the control experiments, whereas with salyrgan alone the output was increased only 2.2 and 26 times, respectively.

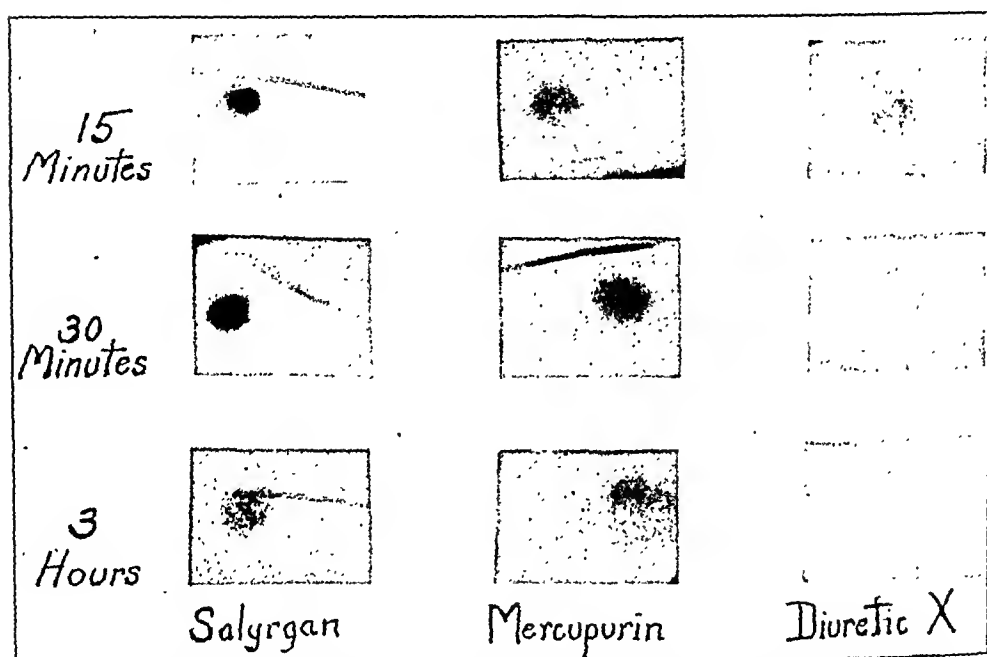


Fig. 1.—X-ray photograph of rabbit's ear at various time intervals following intracutaneous injection of 0.05 c.c. of salyrgan, mercupurin, and diuretic X.

#### SLOUGHS

One of the untoward events in the use of the mercurial diuretics, as noted above, is the occasional production of lesions at the site of injection due to the accidental leakage of the fluid into the skin. We have studied the effect of salyrgan, mercupurin, and diuretic X when injected intracutaneously in animals in doses of 0.05 to 0.1 c.c. The findings may be summarized by saying that the reaction following salyrgan is somewhat more intense than that produced with mercupurin, although both cause a definite necrotic lesion. With salyrgan there is more commonly sloughing of the overlying skin with a resulting open sore, whereas mercupurin usually produces an indurated lesion with a scab formation in the center. The usual precautions, therefore, that are taken to avoid slough formation in the administration of salyrgan need to be observed in the use of mercupurin.

The localization and disappearance of mercury at the site of injection into the skin of a rabbit's ear can be followed by x-ray photographs. This is illustrated in Fig. 1. Immediately after the injection of salyrgan the lesion casts a sharply outlined shadow which is still plainly visible at the end of three hours. With mereupurin the shadow cast just after injection is more diffuse. It tends to disappear promptly so that in three hours there remains only an ill-defined area of increased density.

There was a very striking absence of slough formation in animals following the subcutaneous injection of diuretic X. This preparation in amounts of 0.05 to 0.1 c.c. was injected into or under the skin forty-one times in five rabbits and several times in dogs. In no instance did a definite slough form. The usual lesion produced was an area of erythema and edema 0.5 to 1.5 cm. in diameter, which disappeared within twenty-four to forty-eight hours. A possible explanation of this apparent "protective" action of theophylline is that offered by v. Issekntz and v. Vegh,<sup>9</sup> who felt that the theophylline neutralized the alkaline reaction produced by the hydrolysis of the complex mercury salts in watery solution. From the x-ray photographs taken following the injection of diuretic X (Fig. 1) it is apparent that this substance is not sharply localized at the site of injection as in salyrgan and, moreover, disappears rapidly, indicating that it is absorbed promptly.

#### SUMMARY

The purpose of this communication is to report our observations on the effectiveness of two mercurial diuretics containing theophylline compared with salyrgan. The first of these, merenpurin (novurit), contains theophylline in both free and combined form, the usual dose of 1 c.c. supplying approximately 50 mg. of theophylline. As far as can be judged from its use in a group of sixteen hospital patients with edema of cardiac failure, mereupurin is quite as effective as salyrgan in increasing the output of urine. In specific instances it produced a distinctly greater diuresis, possibly due to the added effect of theophylline. In other instances, there was no evident superiority of the one preparation over the other. It is not a new experience to find one diuretic more effective than another in a given individual, and further use of these drugs will doubtless reveal instances in which better results may be obtained with mereupurin than with salyrgan and vice versa. The other preparation, diuretic X, containing theophylline which merely has been added to an active mercurial diuretic, and which is not available commercially, showed no advantages over salyrgan in six patients. All three of these drugs were equally irritating when injected intramuscularly in patients; in animals, those containing theophylline were less irritating.

Both of the theophylline-containing drugs induced a somewhat greater excretion of urine and chloride in rabbits and dogs than did salyrgan. This fact again would appear to be related to the added effect of theophylline which of itself has a diuretic action in these animals. The somewhat greater

effect of mercupurin and diuretic X in animals emphasizes the inadvisability of judging the efficiency of a diuretic drug solely from its action in experimental animals.

Clinically no preference for any one of these three diuretics, salyrgan, mercupurin, and diuretic X, has been demonstrated.

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#### EXPERIMENTS WITH EVIPAL IN PROLONGED ANESTHESIA\*

A. H. MALONEY, PH.D., M.D., AND R. HERTZ, PH.D., WASHINGTON, D. C.

THE need for a variety of nonvolatile anesthetics for surgery of short duration has been claimant. Evipal, the sodium salt of n-methyl cyclohexenyl-methyl barbituric acid, is one of the newer products of synthetic chemistry designed to meet this need. It occurred to us that this hypnotic agent might also be rendered adaptable for use as an anesthetic for surgery of long duration. This report presents results which we have obtained on the use of evipal for prolonged anesthesia in rabbits and dogs.

*Technic.*—The animals were deprived of food for twenty-four hours preceding the experiment. The usual aseptic measures were employed whenever surgery was performed. An initial anesthetizing dose was given either intra-

\*From the Department of Pharmacology, Howard University School of Medicine.  
Received for publication, March 2, 1935.

peritoneally or by the slow intravenous method to the rabbit and intraperitoneally to the dog. The intraperitoneal method is similar in effect to the slow intravenous method but more uniform in action. In most instances the maintenance doses were given intravenously. A 5 per cent or 10 per cent aqueous solution of evipal was freshly prepared for each seance and administered at body temperature.

Two types of experiments were performed both with the objective of determining the possibilities of effective prolonged anesthesia, using fractional parts of the initial anesthetizing dose either at regular intervals or p.r.n.

In one type no surgery was done. Effective anesthesia was determined by response to pain from pinching the tip of the tail or pricking the animal at various sensitive points with a sharp needle. In the other type various surgical operations were performed. Two typical protocols for the rabbit and two for the dog are presented.

Exper. 1. Evipal 5 per cent solution

Male Rabbit, 2,300 grams

8:45 A.M. 100 mg./kg. initial dose, intravenously

8:50 A.M. Surgical anesthesia

9:05-10:35 A.M. 50 mg. q. ten minutes, intravenously

10:35 A.M. Respiration stopped; 5 mg. pierotoxin intravenously; prompt return of respiration

10:45 A.M. 50 mg., intravenously, respiration twenty-nine per minute

11:00 A.M. 100 mg., intravenously

11:15 A.M. 100 mg., intravenously

11:25 A.M. 50 mg., intravenously

11:35 A.M. 100 mg., intravenously

11:40 A.M. 100 mg., intravenously

11:50 A.M. 100 mg., intravenously

12:00 M. 300 mg., intraperitoneally. Surgical anesthesia continued until after 1:00 P.M. Following morning, living and well

In this experiment good surgical anesthesia was maintained during a period of more than four hours. No surgical measures were undertaken, but observations of the behavior of the animal were recorded. The arterial blood, as observed in the vessels of the ear, became slightly venous in color during the injection of the initial anesthetizing dose. Respiration which at the start was shallow became gradually deeper. In addition to response to pain, changes in rhythm and rate of respiration were cardinal signs either of escape from anesthesia or approach toward the danger zone. As time progressed the periods for symptomatic dosing grew more protracted. When the respiration came to a standstill, while evipal was being injected, pierotoxin was administered antidotally, and we observed a distinct clearing of the color of the arterial blood and improvement in the condition of the animal. In addition there was an absence of the signs of transitory respiratory embarrassment to subsequent evipal injections with no lessening of the degree of anesthesia. This observation suggested the rationality of the use of pierotoxin as a prophylactic measure either just before or following the initial anesthetizing dose of evipal. We tried it in a few cases with good results.

Exper. 7. Evipal 10 per cent solution

Female Rabbit, 3,700 grams

3:20 P.M. 110 mg./kg. intravenously, initial dose

3:25 P.M. Surgical anesthesia

3:25-3:40 P.M. Trachea and carotid artery cannulated and made ready for respiratory and pressure tracings

4:00 P.M. 200 mg. intravenously, total dose—record

4:15 P.M. 100 mg. intravenously, total dose—record

4:35 P.M. 100 mg. intravenously, total dose—record

4:45 P.M. 100 mg. intravenously, total dose—record

5:00 P.M. 100 mg. intravenously, total dose—record  
hysterectomy performed

5:25 P.M. Sacrificed

This experiment demonstrated the successful performance of a prolonged surgical operation under evipal anesthesia. Throughout the exercise there was good anesthesia and good relaxation.

The tracing of the blood pressure record showed a slight inconsequential fall during each fractional injection. The experiment was of two hours' duration.

Exper. 2. Evipal 10 per cent solution

Male Dog, 11.5 kilos

11:45 A.M. 50 mg./kg. subcutaneously

12:50 P.M. Vomits, ataxic, staggers

1:30 P.M. 45 mg./kg. intraperitoneally

1:35 P.M. Narcosis, muscles relaxed

1:38 P.M. Anesthesia

2:00 P.M. 20 mg./kg., deep depression

2:40 P.M. 20 mg./kg., deep depression

3:25 P.M. 15 mg./kg., deep depression

4:30 P.M. Gross reflexes returning. Returned to cage; following morning apparently well; eating. Duration of surgical anesthesia two hours fifty minutes

This experiment demonstrated the slowness of the rate of absorption when evipal is administered subcutaneously, an observation which we have previously reported.<sup>1</sup> A 50 mg./kg. dose, which, given intraperitoneally would cause anesthesia in five to ten minutes, given subcutaneously produced simply stupor, incoordination and staggering. The duration of anesthesia was two hours and fifty minutes and recovery was complete.

Exper. 1. Evipal 10 per cent solution

Male Dog, 19.4 kilos

9:30 A.M. 50 mg./kg. intraperitoneally

9:35 A.M. Prostrate, muscle tone lost

9:42 A.M. Surgical anesthesia

9:50 A.M. 15 mg./kg. intraperitoneally, anesthesia maintained

9:57 A.M. 15 mg./kg. intraperitoneally, anesthesia maintained

10:00 A.M. Femoral vein dissected out, good anesthesia

10:05 A.M. Hypophysectomy by parapharyngeal approach

10:35 A.M. 10 mg./kg. intravenously

11:15 A.M. 10 mg./kg. intravenously

11:40 A.M. 10 mg./kg. intravenously  
 12:10 P.M. Died; hemorrhage into lungs

In this particular experiment a difficult operation was undertaken. An attempt was made to perform a hypophysectomy by the parapharyngeal approach. Unfortunately the animal died from hemorrhage, the anesthetic playing no part. The operation lasted two hours and twenty-eight minutes during which time anesthesia was smooth and uneventful. In Table I we have listed the dosages with effects on the individual rabbits and dogs used in this group of experiments.

TABLE I

NO.	WT. IN KG.	INITIAL DOSE OF EVIPAL MG./KG.	TOTAL DOSE IN MG.	TOTAL DOSE EVIPAL MG./KG.	DURATION OF ANESTHESIA IN MINUTES	REMARKS
A—Rabbits						
1	2.3	100	1030	447	195	Picrotoxin restored arrested respiration; complete recovery
2	2.5	200	875	350	170	Complete recovery
3	2.37	21	375	158	70	Sacrificed following acute experiment
4	2.24	68	914	408	290	Picrotoxin given following initial dose of evipal. Complete recovery
5	2.25	47	490	192	127	Dosed symptomatically. Complete recovery
6	2.27	57	515	236	127	Picrotoxin restored arrested respiration; complete recovery
7	3.7	108	1000	270	120	Picrotoxin given preanesthetically. Trachea and carotid artery cannulated and hysterectomy performed, sacrificed
B—Dogs						
1	10.4	51	2200	113.4	148	Acute experiment hypophysectomy
2	11.5	43	1580	137.4	217	Good anesthesia throughout, survived complete recovery
2(a)	11.5	43	900	78.2	172	Used three days after; survived, complete recovery
3	15	46	1650	110	62	Acute experiment tracheotomy and carotid artery, cannulated, sacrificed
4	13	46	1700	130.7	212	Given 6 mg. picrotoxin while in deep depression, good effect, complete recovery

## DISCUSSION

These figures reveal the fact that the dog shows considerably less tolerance to evipal than the rabbit, the optimal anesthetic dose being 50 per cent less per kilo of body weight. The duration of action of the initial anesthetizing dose is longer and the maintenance doses are correspondingly smaller. This lessened tolerance finds expression in a graded reduction of optimal dosage requirements as one ascends the evolutionary scale in the development of the central nervous system, the recommended anesthetizing dose for man being about 10 mg./kg.

Regarding the effect of evipal on the blood pressure and respiration as revealed by kymographic records: during each injection the pressure in the carotid artery registers a fall below the preceding figure, the degree depending upon the rate of injection. Upon the cessation of the injection, however, it begins to make a gradual return but always stops just short (1 to 3 mg. Hg) of the antecedent level. Synchronously with the fall in blood pressure the respiration becomes slower and shallower, the volume tending to right itself without any significant increase in the rate. When death occurs, it is due to failure of respiration, since the heart continues to beat for a time with gradually diminishing effectiveness while the blood assumes a distinctly venous color. These eventualities could be avoided by use of the slow intravenous method and with the patient in the Fowler position.

In our first rabbit experiment we encountered a stoppage of respiration. Whether or not this condition would have corrected itself we do not know, but spontaneous respiration immediately followed pierotoxin injection. This experience was suggestive of the feasibility of pierotoxin prophylactically. It was tried both before and following the initial anesthetizing dose of evipal with uniformly good results. When so used, it prevents sudden paralysis of the respiratory center, resulting from too rapid intravenous injection. It also prevents the sudden drop in blood pressure from the same cause. We recommend that pierotoxin be routinely available whenever evipal is being employed for purposes of anesthesia. Respiratory depression and fall in blood pressure when not critical, but below the desired therapeutic level, may be overcome by an injection of metrazol, Jackson.<sup>2</sup> In several experiments, not here reported, we have substantially confirmed Jackson's observation using either coramine or metrazol. However, respiratory embarrassment is frequently peripheral, due to mechanical stoppage of the air passage. We frequently found it necessary to keep the tongue forward by continuous traction with a hemostat.

Evipal occasionally produces symptoms of excitement when given subcutaneously or even intraperitoneally in small doses. But when given intravenously the interval between introduction and narcotic action is too short for detectable signs of excitement. In this respect, viz., speed of action, evipal differs from permocton, amytal, and nembutal. In man, due to a longer course of the systemic circulation, the time interval is longer, hence excitement might conceivably occur in rare instances. A few such instances have been recorded. Baetzner<sup>3</sup> attributes such induction excitement to underdosage. The Anesthetic Committee of the Medical Research Council<sup>4</sup> states that such conditions "have been seen only in unusually emotional subjects."

While evipal has established itself as a satisfactory agent for anesthesia of short duration, the above experiments have convinced us that it also has possibilities as an agency in the production of prolonged anesthesia. It was therefore most interesting to note during the preparation of this report that Baetzner<sup>3</sup> has tried fractional doses to prolong the period of anesthesia with no deleterious results.

## SUMMARY

The results of experiments with evipal in the production of prolonged anesthesia on rabbits and dogs are presented.

1. Seven rabbits were kept under good surgical anesthesia with or without operative procedures for periods of 195, 170, 70, 290, 127, 120 minutes, respectively.

2. The total dose of evipal administered fractionally to each rabbit in mg./kg. was 447, 350, 158, 508, 192, 236, 270.

3. Four dogs (one used at two different times) were kept under good surgical anesthesia with or without operative procedures for periods of 148, 215, 172, 62, 212 minutes, respectively.

4. The total dose of evipal administered fractionally to each dog in mg./kg. was 113.4, 137.4, 78.2, 110, 130.7.

5. No untoward after-effects were observed in those animals that were not subjected to surgical procedures.

6. Pierotoxin used before or following the initial anesthetizing dose widens the margin of safety.

7. Gross observations and graphic records are discussed.

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# STUDIES ON THE EFFECT OF THE ADMINISTRATION OF CAROTENE AND VITAMIN A IN PATIENTS WITH DIABETES MELLITUS\*

## I. THE EFFECT OF THE ORAL ADMINISTRATION OF CAROTENE ON THE BLOOD CAROTENE AND CHOLESTEROL OF DIABETIC AND NORMAL INDIVIDUALS

ELAINE P. RALLI, M.D., HAROLD BRANDALEONE, M.D., AND  
THEODORE MANDELBAUM, M.D., NEW YORK, N. Y.

THE presence of carotene in the blood was established as early as 1913 by Van den Bergh and Snapper.<sup>1</sup> In 1919, Hess and Meyers<sup>2</sup> defined the state of an increase of carotene in the blood as carotinemia. Since then the condition has been reported clinically by several observers<sup>3, 4, 5</sup> and particular attention has been called to its presence in patients with diabetes.<sup>6, 7, 8</sup> Rabinowitch<sup>9</sup> has suggested that a high blood cholesterol is related to an increase in blood carotene. The importance of carotene in nutrition has been given prominence because of the fact that it is the outstanding precursor of vitamin A. That carotene will cure vitamin A deficiency has been definitely established by Moore<sup>10</sup> and others. Apparently in the body it is converted by vitamin A by the liver.<sup>10</sup> In view of these facts and because of the tendency for a high blood carotene in the diabetic patient, the relative effect of the oral administration of carotene in oil and of carrots on the blood carotene and cholesterol of normal and diabetic individuals was investigated.

### PROCEDURE

The nondiabetic patients were given constant diets containing no eggs or carrots. At the end of several weeks, during which the blood carotene was determined at intervals, each subject received 20 c.c. of a 0.3 per cent solution of carotene† in oil. In view of the fact that carotene is best absorbed when given with a certain amount of fat, it was administered with 20 gm. of lard spread on two slices of white bread. At the same time the patient was given 200 c.c. of coffee with 30 gm. of glucose. This meal was adopted so that a known amount of carbohydrate could be given the diabetic patients. When carrots were used, 200 gm., cooked to the point of softness, were given with the same breakfast. Blood was then taken at intervals of 1, 3, 5, 10, 24, 30, 48, and 96 hours. After the five-hour specimen, the patient was fed the same type of diet which

\*From the Third (New York University) Medical Division, Bellevue Hospital and the Diabetic Clinics of the University and Bellevue Hospital Medical College, New York University.  
Received for publication, March 19, 1935.

This investigation has been made with the assistance of a grant from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

†The carotene used was supplied by the S. M. A. Corporation and contained approximately 80 per cent beta carotene. Biologically it was found to be equivalent to the S. M. A. crystalline carotene.

he had received during the preliminary period. During the remainder of the observational period he was fed at the usual intervals. Further specimens of blood were taken one week and in some cases two weeks after the original dose of carotene. As it soon became apparent that the blood carotene did not change within the first three hours, the one-hour sample was omitted in subsequent experiments.

Blood serum was analyzed for carotene by the method of White and Gordon<sup>11</sup> in which carotene, extracted from the serum, is matched against solutions of dichromate, so standardized that a 0.2 per cent solution of dichromate represents 100 dichromate units. Blood cholesterols were done by the Bloor colorimetric method,<sup>12</sup> in Cases 1 to 9 inclusive on whole blood and in the remaining cases on blood plasma. Whole blood was used in the earlier curves to avoid withdrawing excessive amounts of blood.

For the study of the effect of carotene on the diabetics, patients were chosen of about the same age groups as the normals, with both moderate and severe forms of the disease. The fasting blood carotene was established on a known diet of carbohydrate, protein, and fat from which carrots and eggs were omitted. On the morning of the test, the patient received the same breakfast and the same amount of carotene in oil or carrots as the normals. In some cases insulin was given before and during the period of observation, in others it was omitted as indicated in the tables. The carotene, cholesterol, and sugar of the blood were determined at the same intervals of time. All the diabetic patients received carrots and carotene in oil, and when possible, both with and without insulin.

Blood sugars are not reported as they bore no relationship to the blood carotene.

## RESULTS

Table I summarizes the subjects studied and gives the average fasting blood carotene and cholesterols before any carotene was administered. The state

TABLE I  
SUMMARY OF DIABETIC AND NORMAL INDIVIDUALS STUDIED

	CASE	NUMBER	SEX	AGE	WEIGHT POUNDS	HEIGHT INCHES	AVERAGE FASTING		DURATION OF DIABETES	DIET IN GRAMS			INSULIN UNITS		
							CAROTENE MG. %	CHOLESTEROL MG. %		CARBOHYDRATE	PROTEIN	FAT	BREAKFAST	LUNCH	SUPPER
Diabetics	A. G.	7	M	47	127	63	.318	272.	7 years	250	65	85	15	0	15
	B. R.	8	M	45	120	68	.216	204.	3 months	200	75	85	20	0	15
	A. C.	9	M	48	140	70	.337	211.	9 months	250	75	85	30	10	25
	M. E.	10	M	40	190	66	.207	241.	3 years	180	65	85	10	0	5
	P. S.	11	M	65	104	60	.337	251.	7 years	180	75	85	0	0	0
Normals	H. P.	1	M	50	151	68	.122	158.							
	P. F.	2	M	45	141	62	.077	176.							
	J. C.	3	M	48	122	65	.122	190.							
	J. P.	4	M	44	148	69	.108	163.							
	S. K.	5	M	22	132	64	.136	172.							
	W. McD.	6	M	34	158	70	.054	165.							

TABLE II  
EFFECT OF 20 C.C. OF .3 PER CENT SOLUTION OF CAROTENE IN OIL ON BLOOD CAROTENE AND CHOLESTEROL OF NORMAL INDIVIDUALS

CASE	MG. PER CENT	FASTING	3 HR.	5 HR.	10 HR.	24 HR.	30 HR.	48 HR.	72 HR.	96 HR.	1 WEEK	NOTES
1	Carotene	.108	.135	.122	.135	.135	.122	.149	.162	.149	.149	
	Cholesterol	168.	161.	180.	192.	183.	173.	161.	156.	175.	147.	
2	Carotene	.068	.108	.108	.095	.108	.108	.122	.162	.135	.108	
	Cholesterol	179.	200.	192.	200.	200.	189.	156.	156.	151.	164.	
3	Carotene	.135	.149	.149	.149	.176	.216	.203	.203	.203	.216	
	Cholesterol	247.	238.	238.	192.	208.	197.	206.	216.	200.	172.	
3	Carotene	.216	.203	.203	.203	.216	.216	.216	.203	.176	.189	7 days after administration of carotene for Curve 1
	Cholesterol	172.	208.	211.	211.	193.	200.	200.	238.	239.	187.	
4	Carotene	.135	.122	.122	.122	.176	.216	.216	.197.	.203	.203	
	Cholesterol	229.	211.	215.	204.	231.	225.	178.	230	166.	153.	
4	Carotene	.203	.203	.216	.203	.243	.243	.243	.230	.216	.216	7 days after administration of carotene for Curve 1
	Cholesterol	153.	196.	215.	199.	222.	220.	199.	234.	219.	200.	
5	Carotene	.122	.122	.122	.149	.189	.162	.176	.203	.162	.108	
	Cholesterol	166.	152.	188.	158.	178.	174.	179.	162.	165.	158.	
5	Carotene	.095	.095	.095	.108	.108	.122	.108	.122	.135	.135	13 days after administration of carotene for Curve 1
	Cholesterol	159.	165.	160.	151.	141.	154.	158.	152.	163.	159.	
5	Carotene	.149	.149	.149	.149	.203	.149	.149	.149	.162	.149	14 days after administration of carotene for Curve 2
	Cholesterol	154.	136.	161.	180.	194.	166.	152.	197.	137.	160.	
5	Carotene	.149	.149	.149	.139	.203	.203	.189	.176	.176	.122	This curve was made after the subject received 1 c.c. of carotene daily for 1 month
	Cholesterol	152.	161.	160.	131.	179.	149.	163.	174.	156.	146.	
6	Carotene	.068	.068	.081	.095	.095	.122	.122	.122	.095	.068	
	Cholesterol	172.	143.	172.	139.	185.	163.	172.	156.	162.	161.	
6	Carotene	.054	.054	.068	.068	.068	.081	.081	.081	.081	.081	14 days after administration of carotene for Curve 1
	Cholesterol	150.	156.	150.	143.	143.	151.	166.	137.	148.	163.	

of nutrition may be estimated from the heights and weights of the patients. In a study involving an essential food, such as vitamin A, it is important that the subject be in good health so that the vitamin or its precursor is not required to satisfy some nutritional deficiency. Tables II and III show the effects, on the blood carotene and cholesterol of normal individuals, of a .3 per cent solution of carotene in oil and 200 gm. of carrots. Tables IV and V give the results on the diabetic patients.

The fasting blood carotene was consistently higher in diabetics than in normals. The one exception to this was Case 14, whose average fasting carotene was .133 mg. per cent. In an earlier series of observations<sup>13</sup> the average fasting blood carotene in normals was found to be .109 mg. per cent, in diabetics .262 mg. per cent. Following the administration of carotene in oil to the nondiabetics there was a rise in the blood carotene which tended to reach a peak after twenty-four hours. In two cases this occurred earlier, in four cases it was delayed to thirty hours or more. At the end of ninety-six hours, the fasting carotene was at or only slightly above the original level in nine of the twelve experiments.

TABLE III

EFFECT OF 200 GM. OF CARROTS ON BLOOD CAROTENE AND CHOLESTEROL OF NORMAL INDIVIDUALS

CASE	MG. PER CENT	FASTING	3 HR.	5 HR.	10 HR.	24 HR.	30 HR.	48 HR.	72 HR.	96 HR.	1 WEEK
1	Carotene	.108	.122	.135	.122	.108	.149	.135	.122	.108	.108
	Cholesterol	150.	168.	188.	177.	161.	185.	185.	178.	217.	179.
2	Carotene	.108	.108	.108	.108	.095	.108	.108	.095	.081	.081
	Cholesterol	145.	186.	203.	182.	167.	200.	189.	179.	208.	164.
3	Carotene	.162	.189	.189	.189	.189	.203	.203	.162	.149	.135
	Cholesterol	211.	203.	227.	213.	201.	202.	214.	205.	194.	185.
5	Carotene	.095	.108	.095	.095	.081	.095	.095	.108	.095	.122
	Cholesterol	177.	153.	161.	151.	147.	165.	147.	152.	144.	166.
6	Carotene	.054	.054	.054	.054	.054	.054	.054	.068	.054	.068
	Cholesterol	177.	147.	158.	143.	151.	150.	168.	160.	156.	172.

The curves for the normals after both carotene and carrots show a certain constancy. In the diabetics the responses were much less regular. Starting at a higher level there was in most of the cases a more precipitous rise, which became evident within the first ten hours in six experiments. This was followed by a definite drop, and in three experiments by an even greater rise at twenty-four or thirty hours. The absolute rise was greater in the diabetic patients after carotene and carrots. This bore no relation to the severity of the disease, but the patients who had the disease longest tended to have the greatest rise in blood carotene after receiving carotene in oil.

Carotene in oil or carrots was administered more than once to certain subjects in the diabetic and nondiabetic groups. In the normals (see Tables II and III), the second administration of carotene was not usually followed by as great a rise in the blood carotene level as it was in the diabetics. In the diabetics (Tables IV and V) the second curve, with the exception of Case 8, was on a higher level than the first and tended to remain elevated at the ninety-sixth hour. Case 7, diabetic, received carotene in oil three times at intervals of two weeks and the blood carotene was further elevated after each administration. A

TABLE IV  
EFFECT OF 20 C.C. OF .3 PER CENT SOLUTION OF CAROTENE IN OIL ON BLOOD CAROTENE AND CHOLESTEROL OF DIABETIC PATIENTS

CASE	MG. PER CENT	FASTING	3 HR.	5 HR.	10 HR.	24 HR.	30 HR.	48 HR.	72 HR.	96 HR.	1 WEEK	ADMINISTRATION OF INSULIN DURING CURVES
7	Carotene	.284	.338	.459	.459	.459	.324	.351	.351	.311	.405	No insulin for one week, 20 units after 7½ hours
	Cholesterol	.274.	.252.	.238.	.255.	.253.	.207.	.217.	.247.	.247.	.227.	
7	Carotene	.567	.594	.450	.513	.459	.567	.567	.513	.594	.513	15 units before fasting, 10 units before 10 hr. specimens
	Cholesterol	.297.	.283.	.317.	.305.	.287.	.342.	.325.	.346.	.291.	.272.	
7	Carotene	.378	.513	.540	.540	.567	.513	.513	.540	.675	.486	No insulin during curve
	Cholesterol	.278.	.250.	.253.	.218.	.294.	.256.	.250.	.266.	.329.	.313.	
8	Carotene	.284		.297	.284	.297	.270	.357	.324	.324	.324	15 units before 10 hr. specimen
	Cholesterol	.214.		.184.	.236.	.260.	.205.	.197.	.231.	.272.	.216.	
9	Carotene	.284		.311	.392	.351	.297	.270	.351	.311	.297	20 units before 5 hr., 30 units before 10 hr. specimens and 10 units at midnight
	Cholesterol	.179.		.225.	.223.	.181.	.221.	.219.	.208.	.212.	.175.	
10	Carotene	.203		.230	.230	.230	.230	.230	.230	.243	.230	8 units before 10 hr. specimen
	Cholesterol	.200.		.203.	.219.	.212.	.195.	.250.			.230.	
11	Carotene	.486		.562	.562	.591	.513	.540		.594	.618	No insulin
	Cholesterol	.105.		.256.	.250.		.223.	.301.		.258.	.291.	
12	Carotene	.297		.297	.324	.338	.311	.311		.324	.311	8 units before fasting specimen
	Cholesterol	.161.		.168.	.227.	.223.	.176.	.210.		.205.	.208.	



similar series of experiments was done on Case 5, a nondiabetic patient. He received three doses of carotene and one of carrots. The second curve was at a definitely lower level than the first. The third started at a higher fasting level, showed a drop and did not rise above the fasting level. The patient was then placed on 1 c.c. of a 0.3 per cent solution of carotene for thirty days after which another dose of 20 c.c. of carotene was administered. In the fourth experiment, the fasting blood carotene was .149 mg. per cent, which was only .027 mg. per cent higher than the original fasting level. This, however, reached a peak of .203 mg. per cent at twenty-four hours and was maintained until the forty-eighth hour, when it began to fall gradually. The fall was slower than in the previous experiments. This curve simulated the type of curve observed in

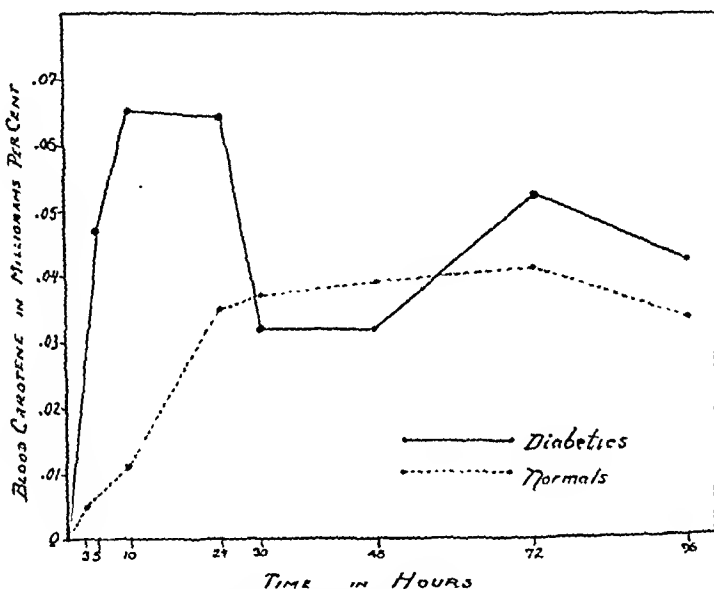


Chart 1.—Curves representing absolute rise in milligram per cent of blood carotene above fasting levels, in normal and diabetic individuals following the administration of carotene. These figures represent the mean values obtained from the figures in Tables II and IV.

the diabetic patients, but to obtain this effect the patient received, in the preceding period in experiments and daily doses, a total of 90 c.c. of carotene in oil.

Concerning the relation of the blood carotene to the blood cholesterol, it was found that prior to any administration of carotene or carrots (Table I) the blood cholesterol in the diabetics was higher than in the normals. There was also a tendency for the high blood carotenes to be associated with higher blood cholesterols. Following the administration of carotene in oil to normals, the blood cholesterol rose slightly and had returned to the fasting level or very nearly so by the end of the curve. If the figures are averaged, there is a tendency for the cholesterol to follow the carotene level, but analyzed individually this is not so. In the diabetics, although the cholesterol level was higher, it did not, following the administration of carotene or carrots, bear any direct relationship to the carotene level and the highest blood carotenes were not

accompanied by the highest blood cholesterols. In this connection it is well to realize that the curves covered a period of ninety-six hours during which time other meals were eaten.

### DISCUSSION

The higher fasting blood carotene in the diabetics and their reaction to the ingestion of carotene may be explained either on the concentration of carotene in the liver at the time carotene is administered or on a concentration of other highly unsaturated substances. In support of the suggestion that the concentration of carotene may be increased in the livers of diabetics the analyses of the livers of 8 diabetic patients and 13 nondiabetics, for vitamin A and carotene is reported (Table VI). The extraction of the liver oil was done by the method described by Moore<sup>10</sup> and the readings, made in a Lovibond tintometer, are reported in yellow units. The antimony trichloride reaction<sup>14</sup> was used for the determination of vitamin A and the results are reported in blue units. Other observers have reported the vitamin A content of human livers, but unfortunately not the carotene content.<sup>15, 16</sup> Of the 8 diabetic patients all but two had more than 1,200 yellow units per 100 gm. of liver. Of these 2, one had hemochromatosis with pigment deposit in the liver, which might have interfered with the absorption of carotene by the liver. Only 4 of the 13 nondiabetic patients had an increased concentration of carotene in their livers, the other 9 having 600 yellow units or less per 100 gm. of liver. These figures, although not conclusive, show a tendency for an increased concentration of carotene in the livers of diabetic patients.

TABLE VI

VITAMIN A AND CAROTENE CONTENT OF HUMAN LIVERS REPORTED IN BLUE UNITS (VITAMIN A) AND YELLOW UNITS (CAROTENE)

CONDITION OF PATIENT	BLUE UNITS PER 100 GM.	YELLOW UNITS PER 100 GM.
Diabetic patient	9,680	9,680
Diabetic patient	14,520	520
Diabetic and Hemochromatosis	1,905	120
Diabetic patient	22,880	1,200
Diabetic patient	84,480	1,920
Diabetic patient	48,400	1,200
Diabetic patient	61,380	1,400
Diabetic patient	48,400	5,000
Nondiabetic patients		
Pulmonary edema	16,560	
Stabbed	8,800	600
Auto accident	35,200	400
Found dead	112,640	560
Pulmonary thrombosis	17,600	480
Apoplexy	56,320	640
Sarcoma, metastases to liver (child)		2,560
Congestive heart failure	10,560	
Stabbed	55,000	1,210
Lobular pneumonia	19,050	3,750
Congestive heart failure	9,900	500
Sickle-cell anemia	19,800	500
Accident	12,100	350
	24,200	150
		1,400



In considering the reasons for increased concentration of carotene in the livers of diabetic patients, two possibilities suggest themselves: the first is a matter of ingestion; the diet of the diabetic might be higher in vegetables and so provide more carotene per se or substances which could be built up into carotene; second, the conversion of carotene to vitamin A may be interfered with in the liver of the diabetic patient.

In this group of diabetic patients four (Cases 7, 10, 11, 12) were clinic patients who had been under observation for periods of from three months to three years. During this time, they averaged 400 to 500 gm. of vegetables daily. The other two patients had, as far as could be established, developed their diabetes in the preceding twelve months, during which time they had eaten an average normal diet. Therefore in four of these cases it is possible to state definitely that the vegetable intake for even long periods of time was not really excessive. It is true that a rise in blood carotene will result from the excessive ingestion of the pigment,<sup>17</sup> but under ordinary circumstances the normal individual can manage to control this, as is shown by the effect of carrots and carotene on the normal group. Furthermore, as shown in Case 5, it was only after receiving a total of 90 c.c. of carotene in oil that it was possible to maintain an elevated blood carotene. Thus the excessive ingestion of vegetables, although a factor in producing an increase in blood carotene, does not seem to offer the most likely explanation.

The second suggestion, that carotene accumulates in the liver of the diabetic because of some interference with its conversion to vitamin A, would mean that a rise in blood carotene could result from the accumulation of carotene in the liver and the consequent inability of this organ to absorb as much from the blood. Moore<sup>10</sup> and others have shown that the conversion of carotene to vitamin A occurs in the liver, and the evidence is reasonably strong that the change is essentially a hydration of the central double bond of the carotene molecule. In 1933 we reported<sup>18</sup> that carotene fed daily to four depancreatized dogs, three received it as carotene in oil and one in two egg yolks, was not converted to vitamin A, as it was in a control nondiabetic dog, previously kept on an A deficient diet. The blue to yellow ratio of the control dog was 587:1. In the four depancreatized dogs it was 11:1, 176:1, 121:1, and 8:1. In two dogs kept on a mixed diet of kitchen scraps it was 1,000:1 and 880:1. Thus the normal dogs, receiving a mixed diet, and the control dog, devitaminized and then fed carotene, had a greater proportion of vitamin A in their livers than did the depancreatized dogs fed carotene.

At the present time we are inclined to feel that the accumulation of carotene in the liver of the diabetic is due to a diminished ability on the part of the liver to convert carotene to vitamin A. This does not mean that hydration of the carotene molecule does not occur at all, but that it may be slowed up sufficiently to allow carotene to accumulate in the liver. When this occurs, a rise in the level of carotene in the blood follows.

#### SUMMARY AND CONCLUSIONS

The fasting blood carotene was found to be higher in 8 of 9 patients with diabetes mellitus than in a group of 9 nondiabetic individuals.

Following the administration of carotene in oil and of carrots, the blood carotene rose sooner in the diabetic patients, the increase was greater and was maintained for a longer period of time.

A second administration of carotene to nondiabetic patients did not result in any greater increase in the blood carotene than did the first dose. In the diabetic patients a second dose of carotene caused a greater increase in blood carotene in all but one case.

To obtain a curve in a normal simulating that in the diabetic patients, it was necessary to administer large amounts of carotene, Case 5 demonstrating this. The prolonged administration of 1 c.c. of carotene in oil to three normal and three diabetic patients resulted in a greater increase in the blood carotene in the diabetic patients and a more gradual fall.

The fasting blood cholesterol was higher in the diabetic patients but bore no absolute relationship to the height of the blood carotene. The blood sugar bore no relationship to the blood carotene.

To explain these results the hypothesis is advanced that there is an increased concentration of carotene in the liver of the diabetic patient due to a diminished ability on the part of this organ to convert carotene to vitamin A, and that this increased concentration interferes with the absorption of carotene from the blood.

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# LABORATORY METHODS

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## THE TECHNIC AND APPLICATION OF SUPRAVITAL STAINING\*

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EDWARD A. GALL, M.D., BOSTON, MASS.

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THE staining of blood preparations for the microscopic study of the living cell is a procedure which has come rapidly to the fore in recent years. It is by no means a new discovery, of course, but its practical application was not fully appreciated until Simpson (1919-1921),<sup>42, 43</sup> Sabin (1921),<sup>34</sup> and Sabin, Doan and Cunningham (1922-1925)<sup>6, 35, 36, 37</sup> utilized the method for the determination of individual cell characteristics and their embryologic development. Since this time a host of workers have demonstrated both the value and limitations of this technic.<sup>21, 45</sup>

Prior to Ehrlich's introduction of the tri-acid stain, the study of the unstained blood in the living state was the only method generally available. Bettmann,<sup>3</sup> Rosin and Bibergeil,<sup>32, 33</sup> and Israel and Pappenheim<sup>22</sup> first used neutral red, a relatively nontoxic organic dye, in their studies of red blood cells. Following this, very little work of moment was done with nontoxic dyes other than the vital staining of cells *in situ* in the living animal by Aschoff and Kiyono.<sup>1</sup> Cowdry, in 1914, first demonstrated the specificity of Janus green for the staining of cellular chondriosomes (mitochondria).<sup>10</sup>

Subsequent to the publications of Sabin, Doan and Cunningham, there has been a progressive increase in the utilization of the warm box and the dyes, neutral red and Janus green, for the purpose of what has been termed supravital staining in the investigation of cellular morphology in disease and health. Technically, this refers to the staining of cells after somatic death (following removal from the body) but before cellular (intrinsic) dissolution. For the most part, the fundamental literature generally available concerning this subject is the production of former students and colleagues of these initial investigators in the field. As such, they have tended to refer for more adequate and detailed description of specific morphology to the original presentations of their predecessors. In those papers in which well-defined descriptive effort is made, too often there is the tendency to leave unsaid that which presumably may be found in the original publications. This naturally makes for considerable difficulty to the novice in this phase of blood study. Furthermore, many of these introductory works are not readily procurable, and many of those which are, vary greatly in the interpretations of the fundamental elements described.

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\*From the Medical Clinic of The Boston Dispensary (Service of Dr. Joseph H. Pratt.), and Department of Medicine, Tufts Medical School.  
Received for publication, February 21, 1935.

For this reason this compilation of the descriptions of the commonly observed human blood cells stained supravitaly in both normal and pathologic states is presented. Personal observation has been utilized as the encompassing influence. Such cells as have not been personally observed are demonstrated by means of analyses of the literature.

*Method.*—For the sake of completeness a brief résumé of the method of staining as outlined by Forkner is appended.<sup>19</sup> For ordinary peripheral blood study the following solutions are necessary:

*Solution I:* 0.125 gm. of vital neutral red<sup>7</sup> ("Toluylene Red," National Aniline and Chemical Co.) is dissolved in 50 c.c. of neutral absolute ethyl alcohol, thus producing a 0.25 per cent solution which is saturated.

*Solution II:* 0.125 gm. of vital Janus green<sup>7</sup> ("Diazine Green," National Aniline and Chemical Co.) is dissolved in 62.5 c.c. of neutral absolute ethyl alcohol. This is a 0.20 per cent solution which likewise is saturated.

Solutions I and II, if well stoppered, may be kept for indefinite periods as stock solutions. They must, however, be kept dust-free by filtration from time to time. Since Janus green and neutral red in combined solution rapidly deteriorate, it is advisable not to combine the two until just prior to application.<sup>19, 35</sup>

*Solution III:* 50 drops of Solution I are added to 10 c.c. of neutral absolute ethyl alcohol. This preparation may be utilized for the staining of blood with neutral red alone.

*Solution IV:* 2 drops of Solution II are added to 3 c.c. of Solution III. This is the solution which is commonly used, since it will identify by stain specificity many more of the characteristic cellular features than Solution III alone. This combination has been used throughout this study, save where more detailed observation of uninhibited motility was to be made.

It has been the author's custom to use ordinary glass slides (75 by 25 mm.). Larger and thinner cover slips (22 by 40 mm.) than those more frequently used have been found to be advantageous in that they produce a thinner and more even spread. The glassware must be scrupulously clean, for the most minute contamination will serve to produce unsatisfactory preparations. Cleansing is accomplished in ordinary cleaning solution (potassium dichromate and sulphuric acid) for twenty-four to forty-eight hours. The glassware is then washed in running tap water for one to two hours, rinsed first in distilled water, next in 70 per cent alcohol, and finally stored in 50 to 70 per cent alcohol until ready for use. At this time slides and cover slips are dipped in clean ether, allowed to dry in air, and then polished vigorously with clean silk. It has been suggested by Sabin that the use of jeweler's rouge will give a higher polish.<sup>36</sup> This has not been found to be appreciably advantageous for routine use.

The slide is swept lightly with a soft camel's hair brush to remove such silk or dust particles as may adhere. It is then lightly flamed and its surface is flooded with Solution III or IV. The dye is next permitted to drain back into its container, the excess stain at the edges is absorbed with blotting

paper, and the alcohol remaining on the surface will evaporate spontaneously. A very thin, smooth film of violaceous pink stain should remain on the slide. A blotchy smear signifies the presence of grease and such a slide must be discarded. Stained slides may be stored for future use in a dust-free container. The precaution should be taken, however, to mark one end of the unstained surface so that identification will be more readily made.<sup>45</sup>

Blood is drawn from the finger or ear by ordinary needle puncture. The skin is prepared for puncture by washing with soap and water, after which the soap is entirely removed with running water. It is then wiped with alcohol and finally with ether. A dry surface is essential. The cover slip is held between the examiner's finger tips, care being taken to allow no part of the flat surface to be touched by skin. The surface is swept lightly with a soft brush and applied cautiously to the freshly risen blood drop. The cover slip is then promptly permitted to drop gently upon the surface of the stained smear. No additional pressure is to be exerted to cause the blood film to spread. If it does not do so spontaneously, the preparation should be discarded. Directly the film has spread, the edges of the cover slip are rimmed with either salvoline or ordinary white vaseline. Here again care must be taken not to press upon the cover slip or cause it to revolve upon the surface of the slide. The preparation is now ready for use.

The slide is placed upon the stage of the microscope which has been previously warmed to body temperature (36 to 38° C.) in a warm box. Fifteen or twenty minutes usually suffice to permit the cells to recover from the trauma of transportation and temperature change, and also to absorb a sufficient amount of dye to allow identification.

A simple warm box may be handily manufactured at a nominal cost. Its four sides are made approximately 24 inches long, 15 inches wide, and 11 inches in height. It is covered above by two lateral boards to which are hinged two central covers which are cut to fit about the tube of the inserted microscope. On the rear wall of the box there are arranged three carbon bulbs which are controlled by a four-way switch so that any number may be used to regulate the temperature within. The walls of the box are insulated by a perforated asbestos lining. The fore side of the box has two lateral arm-holes which are closed by asbestos or rubber curtains and a central peephole to allow for proper approximation of the microscope objective to the slide. At one side there is placed a small hole for the passage of wire for illumination and another for the insertion of a thermometer. The entire apparatus and its production should cost no more than two or three dollars.

Such elementary data are prefixed not superfluously, since much of the critical literature directed at this procedure is resultant upon minor errors in technique.

Care must be taken to avoid pressure of the objective upon the cover slip. It is good policy to examine the smear with the low power lens to determine whether or not there is an adequate spread in the field to be examined. The objective is then lowered into the oil under direct observation through the peephole, raised slightly, and then focused gently.

The best criteria one may adopt for the determination of preparation excellence are the presence of a fairly regular distribution without air bubbles, the regular contours of the red blood cells, the absence of nuclear staining, and the continued motility of the active leucocytes. Mitochondria are stained by Janus green, a brilliant blue green. Neutral red varies its staining characteristics with the pH of the structures stained, from light yellow to deep maroon.

*Morphology.*—*Red Blood Cells:* *Erythrocytes* appear morphologically as in the unstained state, occasionally greenish in color, but more often exhibiting a straw yellow tint. There is no inherent motility. Fluid currents, however, will carry the cells smoothly across the field, changing their shapes as they adhere transiently to static objects. Thereafter the cells rapidly resume their rounded form. Older preparations exhibit clumping, some disintegration, and general crenation. The normal cell border is distinct, smooth, and rounded but the central pallor is less often observed than in unstained preparations. Neutral red and Janus green will both stain reticular substance in less mature cells, but only in a concentration which would be too toxic for routine use.<sup>36</sup> The same applies to parasitic inclusion bodies. In most bloods, however, small globular, refractile, homogeneous bodies staining bright red may be observed to overlie or lie within the stroma of an occasional red cell. There is usually a single body in such a cell, but as many as 3 to 6 have been observed. These bodies frequently dance actively within the bounds of the cell border and are rarely seen beyond it. It is possible that they are extruded bits of oxyphilic hemoglobin or merely artifacts. They do, however, appear in approximately 0.1 to 1.0 per cent of red blood cells in normal bloods, but have been observed as high as 10 to 15 per cent in spreads from patients who have evident bone marrow stress (myelogenous leukemia, chronic hemorrhagic anemia, and metastatic bone cancer). Similar globules have been described previously by Isaacs.<sup>49</sup> Not infrequently there are present in the same erythrocyte, apparently joined to the globule, small, fine, granular shreds stained greenish blue. Less often this substance occurs alone. There is no evident relationship of the latter to the specific material within the reticulocyte.

The *Megaloblast* has never been observed in peripheral blood by the author. It is said to be a nonmotile cell with a hazy, tenacious border, considerably larger than the mature erythrocyte. The nucleus, usually round, may be oval or indented and is centrally placed. It has a slim, fine border and a sparse meshwork of chromatin in which may be observed a large vacuolar nucleolus and rarely two. The nucleus is quite large in proportion to the size of the cell and often covers as much as two-thirds of its flat surface. Mitosis has been recorded. Characteristic is the earliest faint glow of hemoglobin in the cytoplasm. The latter is nongranular and homogeneous save at the border of the nucleus where some mottling may appear. No structures of coarse, rod-shaped and coccoid mitochondria scattered without pattern persist and after some fifteen minutes the color is lost, and the mitochondria

TABLE 1

Size	ERYTHROBLAST	NORMOBLAST	MONOBLAST	MYELOBLAST	MYELOCYTE	MONOCYTE	LYMPHOCYTE	LYMPHOBLAST	POLYMORPHO- NUCLEAR NEUTROPHILE	CLASMATOCYTE	EOSINOPHILE	PARAPHILE
8-12 $\mu$ Nonmotile	7.5-10 $\mu$ Nonmotile	9-18 $\mu$ Nonmotile	10-12 $\mu$ Nonmotile	8-12 $\mu$ Nonmotile	10-15 $\mu$ Nonmotile with f. G. Motile with N. R. alone. Slow; from one position in 10-15 min- utes. Surface film rolling	6-14 $\mu$ Rarely motile with f. G. Intermediate form usually. Slow rate. Flows through constrict- ions with nucleus aloft 37° C.	8-10 $\mu$ Round Nonmotile	8-12 $\mu$ Actively ameboid. Irreg. course 34 $\mu$ per min- ute at 37° C. Persists	10-80 $\mu$ Motile as Mono- boid	8-12 $\mu$ Motile. Ame- boid Sl. slower rate than poly but immobile in 30-45 min- utes	7-10 $\mu$ Motile Rate as high or higher than poly Persists longer than eos. but not as long as poly	
Border	Rounded Distinct	Round or oval Distinct Bulge of ex- truding nucleus	Fine and smooth Irregular Blebbie excres- cences rather than filiform pro- cesses (Latter occa- sional)	Round Thin and ten- acious No processes	Round or oval Thin Distinct No pseudopodia	Irregular Faint spike-like processes waving, and blebbie pseudopodia	Round or oval Distinct No pseudopodia Constriction only when motile	Distinct No pseudopodia	Irregular Repeated pseu- dopod forma- tion May stretch out to great length	Smooth Instant form and blebbie pro- trusions	As in poly.	As in poly
Cytoplasm	Homogeneous Yellow tint (11b). Gel	Homogeneous Full comple- ment of hemoglobin Gel	Slightly foamy Ground-glass Gel	Ilazy Slightly mottled Gel. No move- ment	Homogeneous Sol in late "Gel" Myelocyte	Foamy, ground- glass Clear surface film Included bodies move about Sol	Clear and glassy Homogeneous Yellow tint in younger forms Sol	Clear Homogeneous Faint yellow tint Gel	Homogeneous and clear Ecto- and endoplasm streaming Granules rare in ectoplasm Sol	Abundant Clear Sol	Clear and homogeneous Well-defined ectoplasm Sol	Clear and homogeneous No ecto- or endoplasm Sol
Nucleus	Eccentric Small $\approx$ cyto- plasm. Chromatin dense Single nucleolus Occas. mitosis	Eccentric or ex- truding Round Chromatin very dense and structureless	Oval or indented (Occas. other shape) Chromatin poorly developed, hardly visible meshwork 1-2 nucleoli occas.	Round or in- dented As blast Membrane more distinct	Round or in- dented As blast Membrane more distinct	Eccentric Round-horse- shoe-bizarre Membrane dis- tinct Chromatin loose regular mesh Soap-suds ap- pearance	Large Lymph: Eccentric Thick mesh concent. at membrane Chromatin loose coarse Round, oval or indented Small Lymph: Central Takes up most of cell	Small and round Eccentric Membrane fine, distinct Chromatin loose mesh, granular 1-2 nucleoli	Poly-nuclear. Shape com- pressed Drags behind with cell flow	Round or oval (never horse- shoe) Central Chromatin faint, scattered clumps May be 2-3 nuclei	Usually bino- bate Dragged as in poly	Rounded or lobed do not stretch. At fore end of cell with mo- tion

TABLE I—Continued

ERYTHROBLAST	NORMOBLAST	MONOBLAST	MYELOBLAST	MYELOCYTE	MONOCYTE	LYMPHOCYTE	LYMPHONLST	POLYMORPHO- NUCLEAR NEUTROPHILE	CLASMATOCYTE	EOSINOPHILE	BASOPHILE
Occasional Superimposed red globules (Refractile)	A <sub>2</sub> in erythro- blast	None Pronocytic has few brick red clumped near centro- sphere (Fixed here)	None	A = 3-10 scat- tered B = 50-100 C = Full com- piment Motion only in late "C" Refractile, bright red	40-80, vary from dust size to vacuole Nonrefractile Fresh brick red Diffusely scat- tered; occas. rosette All same color	Small: Has few (3-5), small size, scattered Large: Has more—large gran- May clump Bright red—re- fractile	None	Stream and dance Fill endoplasm Tiny, round yellowish pink— refractile Occas. nonre- fractile vacuole	Large numbers Move actively with no pat- tern Vary from dust particles to R, B, C. size Color varies from scarlet to maroon Nonrefractile	Stream and dance Oval, all same size Larger than poly- Bright yellow Refractile	Stream and dance Fewer and smaller than eos Round, vary in size Deep maroon Nonrefractile
Small coccoid Gathered loose- ly at nucleus, often to one side	Fewer and smaller than erythroblast Sharply limited to one side of nucleus	Tiny, coccoid Diffusely scat- tered Occas. small clumps or large clump in nuclear hof	Slender threads and commas Constant size in same cell Diffusely scat- tered	A = Fine, coc- coid, fill cell B = Fewer, mixed with N.R. C = Few at cell periphery only	Myriads; fine, dust-like coc- coid Scattered dif- fusely	Many (10-60). Coarse, coc- coid or rod Tend to clump at one side of nucleus with few scattered elsewhere	Short, plump rods scat- tered, more often closely adherent to nucleus Numerous	Few and fine Obscured by N. R.	Rare, if any Nonrefractile	Rare	Rare, occas. small clump of tiny coccoid
None	None	None	None	None	Occasional (rare) refrac- tile mahog- any granule	Refractile, ma- hogany granules (1-3) in 34%	Occas. (rare) refractile, mahogany granule	None signifi- cant	Unstained vacuoles, debris, etc.	None	None
None	None	None	None	None	Phagocytic for particles Lie anywhere in cytoplasm peripheral to rosette	-----	-----	Phagocytic for debris, bac- teris, etc.	Phagocytic for particles or whole cells. Injected par- ticles come to lie at first, close to nucleus	-----	-----

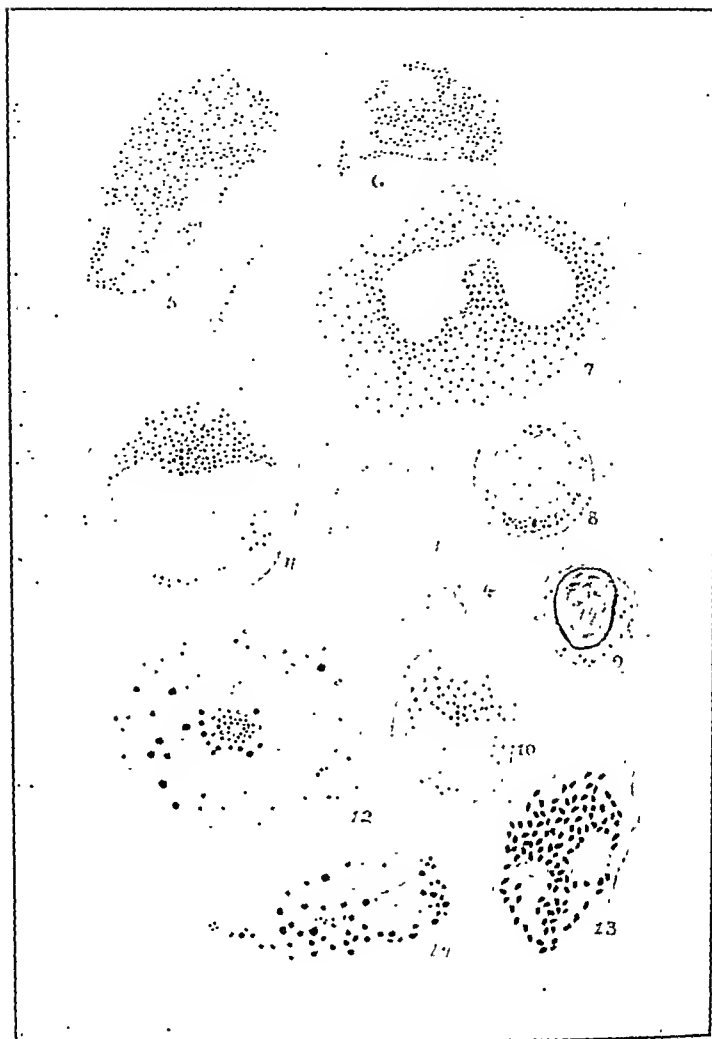
Neutral Red

Janus Green

Unstained  
Bodies

Phagocytosis





**Plate I.—1, Erythrocyte:** This cell is introduced for size contrast, the remaining cells being drawn to scale. Stained, it exhibits the straw yellow of hemoglobin with a faint greenish tint imparted by Janus green. **2, Platelet:** Multiangular in configuration, this element contains a few scattered granules which stain variously with Janus green and neutral red. **3, Polymorphonuclear Leucocyte:** The nucleus is observed to be dragging behind in the motile cell. Specific granules fill the endoplasm and when stained appear as small, round, refractile, yellowish pink bodies. A clear ectoplasmic rim is demonstrated. **4, Polymorphonuclear Leucocyte:** This cell exhibits a long, trailing pseudopod with a bulbous tip which may break off and resemble a platelet. **5, Nonmotile Leucocyte:** The nucleus is large, structureless, and evidently edematous. It is surrounded by many unstained, dark refractile granules which are scattered diffusely. **6, Small Lymphocyte:** There is a clear, homogeneous cytoplasm which contains typically clumped, short, rod-shaped mitochondria. A refractile "mahogany" granule appears opposite the shallow bay of the nucleus. **7, Effete Lymphocyte:** The nuclear membrane is fairly broad and refractile. Chromatin is coarse and smudgy. Hazy cytoplasm contains a few scattered, unstained refractile granules. **8, Intermediate Lymphocyte:** The clear cytoplasm contains many loosely clumped coccoid mitochondria among which may be found a fluted appearance. The nucleus is kidney-shaped and its chromatin exhibits a fluted appearance. **9, Large Lymphocyte (As seen in glandular fever):** There is moderately coarse chromatin within the large bean-shaped nucleus. Mitochondria are clumped about the nucleus. Small brick red bodies here form a true rosette within the nucleus. **10, Eosinophile:** The clear cytoplasm contains numerous bodies with the same staining intensity but varying size. The cytoplasm exhibits a ground glass appearance and has a foamy border with fragile, wavy pseudopodia. Tiny mitochondria are scattered generally. **11, Basophile:** The bilobate nucleus has a wavy border, and its chromatin is loosely meshed. **12, Eosinophile:** The bilobate nucleus is observed dragging behind in the motile state. Its cytoplasm is clear and the endoplasm contains large, oat-shaped, yellow refractile granules. **13, Basophile:** Deep maroon, nonrefractile, round granules are seen scattered about the cytoplasm. They vary moderately in size and staining intensity. There are a few small clumps of tiny mitochondria. The bilobate nucleus is folded and seen at the fore end of the cell.

appear fewer in numbers, and for the most part as indefinite, nonrefractile, smudgelike, unstained bodies. Bone marrow studies have been the basis for the greater part of these observations.<sup>12, 14, 34, 35</sup>

The *Erythroblast* is approximately the same size as the previously described cell. It, too, as are all erythrogenic entities, is nonmotile. Its border is more distinct, and its nucleus, smaller in proportion to the amount of cytoplasm, is eccentrically placed. Mitosis is occasionally observed. There is increased chromatin density and the nucleolus is often obscured. The cytoplasm is entirely homogeneous and the yellow tint of hemoglobin is appreciably deepened. It is at this stage that red globules and their accompanying green blue shreds are first demonstrated. Other than these there is no structure stained with neutral red. Mitochondria are generally smaller, coccoid, and no longer diffusely scattered, but are loosely gathered about the nucleus, more often on one side. They now hold Janus green persistently.<sup>12, 14, 34</sup>

The *Normoblast* is very slightly larger than the mature cell. Its border is distinct and contains within its bounds a full complement of hemoglobin. The nucleus is now quite dense and even in the unstained state presents itself prominently. It is eccentrically placed and is observed to lie beside the cell membrane, often causing it to bulge. It may even be seen extruded into the surrounding medium. The cytoplasm is homogeneous and a significant percentage demonstrates the red globules described above. Mitochondria are much smaller in size and fewer in number. They are sharply localized to one side of the nucleus, and when this is finally lost to the cell, the clump of blue granules may remain visible for a short time.<sup>14</sup>

*Leucocytes:* The *Lymphocyte* is a vari-sized unit. Three forms are found in peripheral blood: the large, which is approximately 10 to 15  $\mu$  in diameter; a small one, approximately the size of a red blood cell; and a form intermediate between the two.<sup>9, 45</sup> The larger cell is, in the moist preparation, the less mature, according to Wiseman,<sup>47</sup> though it is generally believed that size is no conclusive criterion of age. Certainly, if the presence of neutral red bodies is any evidence of lymphocytic maturity, as some claim, size may not be utilized as such a standard. At all events, the *large lymphocyte* is a round, nonmotile cell with clear, glassy, homogeneous cytoplasm occasionally exhibiting a faint yellowish tint.<sup>47</sup> The nucleus, centrally or slightly eccentrically placed, is rounded or indented. It is quite large in proportion to the cytoplasm and contains a fairly thick meshwork of fine chromatin strands which do not aggregate peripherally or elsewhere. The mitochondria are short rods, relatively coarse, and tend to be clumped at one side, often opposite or overlying the hof of the nucleus. There are a few scattered chondriosomes in the paranuclear zone, and occasionally a small subsidiary clump opposite the main aggregation. Neutral red bodies may or may not be present. When they are, rarely exceeding 4 to 10 in number, they stain a deep red and appear as small, refractile globules about the same size as the mitochondria. They are scattered without pattern throughout the cytoplasm, often overlying the nucleus. Both Janus green and neutral red stained bodies may be observed to move with irregular activity within the stroma of the cell. In certain

states, such as infections mononucleosis, wherein pathologic stimulation of the lymphocytopoietic organs obtains, many more neutral red bodies may appear and congregate in the region of the nuclear hof, displacing the mitochondria, and closely resembling the rosette of the monocyte.<sup>21, 29, 31</sup> Certain other criteria, as will be described elsewhere, serve to distinguish the cell.

The *Small Lymphocyte* is a rarely motile, rounded cell with a distinct border. It possesses a clear, untinted, homogeneous cytoplasm and a rounded, centrally placed nucleus. In a number of cells the nuclear contour is irregular and may even be fragmented, resembling the nucleus seen in the Rieder lymphocyte of the fixed smear.<sup>25</sup> In pathologic lymphocytic conditions the proportionate percentage of these forms increases. This is not so well demonstrated in fixed preparations. The chromatin network is denser than that of the previously described cell, the strands are quite coarse, and there is some condensation at the nuclear periphery. Mitochondria are fairly coarse and may be clumped at one side or encircle the nucleus with a single or double row. Neutral red bodies are rare but may occur up to 4 to 6 per cell.<sup>9</sup> This small form is the lymphocyte most frequently observed in normal blood.

The *Intermediate Lymphocyte* has a rounded or slightly irregular border which is definitely delimited. This cell, when stained with neutral red alone, has a characteristic motility. Its rate is slower than that of the polymorphonuclear, averaging about 4 to 15  $\mu$  per minute, and it progresses without pseudopod formation.<sup>25</sup> Its nucleus is found at the fore end of the moving cell, the cytoplasm and contents dragging behind and passing through static constriction rings (i.e., the rings retain their relative position to objects external to the cell while the cytoplasm passes through them, constricting as it does so and regaining its breadth afterward).<sup>24</sup> The cytoplasm has the clear, homogeneous consistency specific for the lymphocyte and is not tinted. The nucleus is relatively smaller in proportion to the cell bulk than in other mononuclear cells. It may be rounded, but is more often oval or kidney-shaped. Its chromatin content usually resembles that of the small form but may have a linear configuration which affords it a fluted appearance. There is, too, observed the "radkern" or wheel spoke arrangement as seen in the so-called plasma cell of the fixed smear. Mitochondria are similar in contour, size, and distribution to those of the other lymphocytes. Neutral red bodies likewise appear in this type but in a larger number of the cells than in the small form. They, too, may assume the pseudorosette appearance of the large lymphocyte.

There are not infrequently observed in blood preparations, particularly those from young children and patients with pronounced lymphocytosis, the "effete lymphocytes" described by Sabin.<sup>18, 36</sup> These present either a free nucleus or one surrounded by an ill-defined, hazy, slightly opaque remnant of cytoplasm. The nuclear contour is round and its border is sharp, thickened, and refractile. The chromatin is dense, smudgy, and vague in outline. External to the nuclear membrane either free in the plasma or imbedded within the cytoplasmic remnant are a few rounded, unstained, refractile bodies.

In some 34 per cent of small and intermediate lymphocytes, in rare monocytes, but in no other white blood cell there are observed one or a pair

of round, dark, mahogany colored, refractile bodies about 0.10 to 0.25  $\mu$  in diameter lying in the cytoplasm. Usually they occur opposite the bay of the nucleus. These bodies resemble morphologically certain of the cell inclusions described by Auer within large mononuclear cells in acute leucemia.<sup>2</sup> They have, however, been observed by the author in all bloods examined, their numbers varying in certain disease processes. Their significance is not at this time discerned. They are visible in the unstained cell but rarely persist in the fixed smear.

The *Lymphoblast*, about the size of the intermediate lymphocyte, is a rounded, nonmotile cell with relatively deficient, clear, yellowish cytoplasm.<sup>9, 47</sup> Its nucleus is small, round, and eccentrically placed. It possesses a fine, distinct nuclear membrane and a loose, thin meshwork of granular chromatin occasionally aggregated at the periphery, within which are found one or two darker nucleoli.<sup>25</sup> The mitochondria are short, plump, fairly coarse rods, which may be scattered throughout the cytoplasm, but more often assume a characteristic position close and adherent to the nucleus.<sup>9</sup> They are not clumped but tend to encircle the central structure. Neutral red bodies do not appear.

The *Polymorphonuclear Leucocyte* in the active state is an irregularly shaped cell about 12 to 23 by 6 to 10  $\mu$  with a distinct border which constantly changes its contour as a result of pseudopod formation.<sup>23</sup> The cell is rarely still but moves about in a circinate ameboid fashion. In the perfect preparation at body temperature its course is constant, in that for long periods of time the fore end remains persistently ahead. Frequently, as the cell proceeds, it may permit a long strand of cytoplasm to remain stretched out behind it. This process often has a rounded, somewhat bulbous tip. With continued movement the narrow pseudopod is withdrawn into the cell body, but occasionally its tip may break off and lie free in the plasma. It is these bits of protoplasm which have often been mistaken for motile platelets.

The rate of locomotion of the polynuclear cells has been estimated by McCutcheon to be approximately 34  $\mu$  per minute at 37° C. and this may increase moderately up to 40° C.<sup>26, 27</sup> Above this temperature the cell becomes static and there are alternately projected and retracted small ineffective pseudopodia. This is the so-called "prickle cell" of temperature paralysis although similar phenomena are noted at body temperature following incompatible transfusion.<sup>36</sup> Eventually, after swirling about seemingly wildly for a brief span, the cell will assume a rounded shape, all cytoplasmic movement will cease, and the nucleus will begin to stain, indicating cell death. At lower temperatures movement is much more sluggish, and below 15 to 20° C. the cell will be observed to "round up." Cell death will not, however, occur, for if after several hours the preparation is again brought to body temperature, normal appearance and movement will be resumed.<sup>23, 27</sup>

The polymorph nucleus has a fine, distinct border and a moderately coarse chromatin. It more often follows passively in the latter portion of the cytoplasm as the cell proceeds and may be elongated or compressed into all manner of bizarre shapes. The chromatin takes no dye normally. Staining of

nuclear structure is a definite indication for discarding the preparation, since cell death is imminent.<sup>37</sup> The cytoplasm is homogeneous and divided into definite endo- and ectoplasm.<sup>5</sup> The dividing line is well demarcated by the failure of neutral red bodies to proceed other than singly into the ectoplasm.

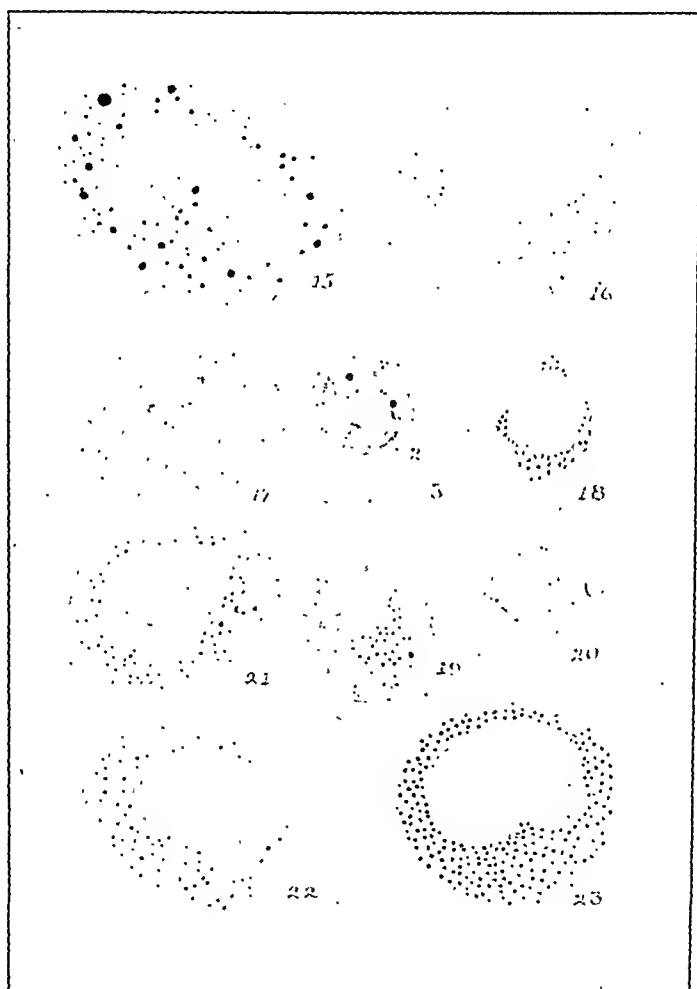


Plate II.—2, *Erythrocyte*: This cell contains two bright red, small, refractile globules. There are also two finely granular, greenish shreds. 3, *Platelet*: The substance of this element has a yellowish tint. There are a few fine, bluish green granules scattered within it. 15, *Monocyte*: This is the usual appearance in normal human blood. It is similar to Cell No. 12 except that neutral red bodies are scattered without rosette formation. 16, *Monoblast*: Blunt, rounded excrescences are seen on the cell surface. Chromatin is faint and fine, and the nucleus contains a single nucleolus. The cell is similar to Cells No. 12 and 15, but contains none of the structures stained with neutral red. 17, *Myeloblast*: The nucleus has a thin, almost imperceptible nuclear membrane and contains a fine chromatin meshwork. There are three nucleoli present. The cytoplasm exhibits some mottling in the perinuclear zone and there are many thin, elongated mitochondria scattered diffusely. 18, *Lymphoblast*: The cytoplasm is homogeneous and has a faint yellowish tint. The nucleus has a definite membrane, has an irregular chromatin mesh, and contains a single nucleolus. Coarse rod-shaped mitochondria are grouped close to the nuclear border. 19, *Erythroblast*: The homogeneous cytoplasm of this cell has the straw yellow tint of hemoglobin. The nucleus is small and eccentric and mitochondria are grouped to one side. A superimposed, red globular body is seen. 20, *Normoblast*: The nucleus appears at the periphery of the cell. Its chromatin is quite dense. The cytoplasm contains a full complement of hemoglobin and a few fine mitochondria clumped at one side of the nucleus. A small amount of greenish material is present. 21, *Myelocyte "A"*: Among them are a few neutral red bodies staining a brighter red than 22, *Myelocyte "B"*: This cell is similar to No. 21 except that more neutral red bodies are present and mitochondria are fewer in numbers. 23, *Myelocyte "C"*: Here, there is present the full adult complement of neutral red bodies. Mitochondria are fewer in numbers and appear only at the cell periphery.

Streaming actively and dancing about within the endoplasm, which they fill completely, are numerous, tiny, round, yellowish pink, refractile granules which are specific for this polymuclear cell. These bodies rush about continuously in every direction and occasionally dart into the ectoplasm but are rapidly withdrawn again into the compact mass. Frequently they form the core of a pseudopod which is otherwise perfectly clear ectoplasm. In some of the neutrophils large, nonrefractile vacuoles taking a deep red stain are observed among the streaming granules.<sup>45</sup> These usually appear within cells which have been in contact with the dye for one and a half hours or longer and may grow to the size of a nuclear lobe. Usually one appears, but there may be four or more. There are a few fine mitochondria present, but in the double-stained cell these are more often obscured by the other cytoplasmic contents.

The active leucocyte may not infrequently be observed to "round up" and remain still for a few seconds, but shortly it will again project pseudopodia and proceed on its course. In overstained preparations and near the edges where drying occurs this assumption of globular form is quite general and such areas are therefore significantly to be avoided. The author notes the number of these forms counted and utilizes their percentage as an indication of preparation perfection. These same drying regions, and occasionally freshly drawn blood, contain cells which have been termed by Sabin "nonmotile leucocytes."<sup>36</sup> She claims that at least in the rabbit these occur in cyclic showers throughout the day and may reach a level as high as 16 per cent of the total white blood count. This has not been conclusively confirmed in the human being. These cells exhibit large, swollen, structureless nuclei surrounded by scattered aggregations of unstained, slightly refractile granules. There are no well-defined delimiting membranes. No motility, other than some brownian movement of the granules, is observed.

The *Myeloblast* is an immobile, rounded cell with a thin, rather tenacious border. It is about 10 to 12  $\mu$  in diameter. The cytoplasm is rather hazy, somewhat mottled in appearance, and is evidently in a gel state, for no movement of its contents is noted. The nucleus is rounded or rarely indented, and it is eccentric in position. The nuclear membrane is smooth, even, and exceedingly thin, so thin, in fact, that it may be almost imperceptible.<sup>15</sup> Fine chromatin forms a loose meshwork with no condensation except about the nucleoli, which are two to four in number. Occasionally in subacute or acute leucemia, the nucleus may assume an irregularly indented contour which resembles the true Rieder cell. Indentations may progress to actual fragmentation of the nucleus. Mitosis is sometimes observed.<sup>9</sup> There are no neutral red bodies in this cell. Mitochondria are extremely fine, slender threads or commas and are diffusely scattered throughout the cytoplasm. They vary in size from cell to cell but within the same cell their size is quite constant. Rarely, they form a clump opposite one side of the nucleus.

*Myelocytes* are likewise immobile, rounded, and some 8 to 12  $\mu$  in diameter. They are subdivided into three arbitrary groups (A, B, and C), indicative of progressive maturity.<sup>9</sup> All contain a rounded or indented eccentric nucleus,

resembling closely that of the myeloblast, but losing the nucleoli as ripening occurs. The nuclear membrane, too, becomes more distinct. The cytoplasm is homogeneous and the mottling of the earlier stage is no longer observed. In the myelocyte "A" there are not more than 3 to 10 neutral red bodies. These are scattered without pattern throughout the mitochondria which still retain the same distribution as observed in the blast form. Occasionally they may clump in the region of the centrosphere, but this is rarely the case. These neutral red bodies are similar in shape, size, and refractility to the specific granules of the adult cell but stain with a deeper, bright red tint. The myelocyte "B" is like the "A" form except for an increase in the number of neutral red bodies. These, numbering 50 to 100 or more, are scattered throughout the mitochondria which now begin to decrease in numbers and are apparently being displaced toward the periphery of the cell. The myelocyte "C" contains a full complement of neutral red bodies which now fill the cytoplasm and compress the few remaining, minute mitochondria to the cell border. At this time fluidity occurs and movements of the cytoplasm ensue. Shortly afterward the nucleus indents further and ameboid movement is initiated so that the cell becomes the almost mature band form.

The *Monocyte* is a fairly large cell, 10 to 15  $\mu$  in diameter, barely, if at all, motile when stained with Janus green.<sup>9, 17, 21</sup> In preparations dyed with neutral red alone, it exhibits a slow, sliding motion, leaving one position entirely only after some five or ten minutes. Characteristically, it progresses by means of a rolling movement of the faint, irregular surface film which is followed more sluggishly by the deeper cell elements. Occasionally the cell appears to advance almost by shifting of cytoplasmic layers in successive depths. The border of the cell in the double-stained preparation is barely visible and irregularly broken by transient flat broad and spikelike protrusions. The latter wave about in the fluid currents and may either be withdrawn, break off, or merely disappear. Phagocytosis occurs by simple engulfment of debris and cell particles which lie in the neighborhood of the active monocyte. This cell apparently has a predilection for fragments rather than whole structures and rarely is an intact erythrocyte observed within it. Engulfed fragments are described as coming to lie characteristically at the outskirts of the rosette (vide infra).<sup>37</sup> As a matter of fact, such material as is phagocytized may be found in any portion of the cytoplasm.<sup>21</sup>

The protoplasm of the monocyte has a foamy, ground glass appearance with a mottled, indefinitely outlined surface film. It is evidently in sol form for enclosed bodies are observed to move about in a sluggish, jerky fashion, occasionally darting for a short distance. Streaming, as seen in the polynuclear cell, does not occur here. The nucleus is always eccentrically placed and may be rounded, indented, horseshoe-shaped or even resemble a petaled flower. In certain abnormal states fragmented or double nuclei may appear.<sup>13</sup> The nuclear membrane is fine, occasionally wavy, but quite distinct, and the chromatin content forms a very delicate, loose meshwork which affords a fine, sudsy appearance. Within the cytoplasm are observed numerous (40 to 80) neutral red bodies varying in size from minute dustlike granules to large

bulging vacuoles several  $\mu$  in diameter. All these bodies take the neutral red stain with exactly the same intensity, a nonrefractile, fresh brick red.<sup>44</sup> The number of larger vacuoles varies directly with the age of the preparation. In the rabbit and guinea pig, but much less often in the human being, there has been described a characteristic development of small neutral red vacuoles of uniform size in concentric rows (2 to 5) about the clear area of the centrosphere opposite the nuclear hof. This has been termed the rosette.<sup>4, 9, 20</sup> The epithelioid cell of tuberculosis has been described by Sabin, Doan, and Forkner as developing from this form of monocyte by condensation and proliferation of the rosette vacuoles with diminution of their individual breadth.<sup>35, 39, 41</sup> As the rosette becomes larger and more compact, the cell enlarges, but no other significant morphologic change occurs. Scattered throughout the cytoplasm, peripheral to the rosette when it is present, are myriads of fine, dust-like coccoid mitochondria. These, along with the smaller neutral red bodies, tend to give to the cytoplasm its ground glass appearance.

The *Monoblast* is somewhat larger than the adult form and has no gross motility. Its border is fine, irregular, and exhibits many bleblike excrescences on its surface. There are fewer of the fragile spikelike protrusions. The cytoplasm is less foamy than that of the monocyte but the ground glass appearance is very similar. The eccentric nucleus is oval, indented, or occasionally horseshoe-shaped. Its chromatin is so poorly developed that its structure is hardly visible in the unstained state. Occasionally one or two nucleoli may be visualized. Small, blunt, coccoid mitochondria are abundantly scattered throughout the cytoplasm. Infrequently they form several small or one large clump in the nuclear indentation. In the early blast form there are no neutral red bodies, but as the cell progresses to the stage known as the premonocyte, some few neutral red bodies appear in the region of the centrosphere where they remain fixed. Their staining characteristics are similar to those of the neutral red bodies in the adult cell.<sup>9</sup>

The author has not identified *clasmatoocytes* as such in the peripheral blood. They are reputedly large cells, 10 to 80  $\mu$  in diameter, actively motile in a manner similar to that of the monocyte.<sup>30, 37, 39, 44</sup> Carrel and Ebeling describe the cell as possessing an undulating membrane which acts as the propelling agent.<sup>5</sup> The cell is highly phagocytic and will engulf as many as twenty, thirty, or more whole red blood cells. Phagocytized objects characteristically come to lie adjacent to the nucleus initially.<sup>37</sup> The cell contour is relatively smooth but may exhibit transient bleblike and filiform pseudopodia.<sup>48</sup> The cytoplasm is abundant and evidently has no characteristic feature. The nucleus is round or oval, fairly large, and located near the center of the cell. Though large by contrast with the nuclei of other leucocytes, it is relatively small in proportion to the cell bulk. Indentation never progresses to the production of the horseshoe nucleus, but two or three nuclei have been observed in stimulated cells. The chromatin is very faint and is arranged in small scattered clumps. Neutral red bodies are present in large numbers, moving about constantly but exhibiting no regular distribution. There is no rosette. These bodies vary in size from dustlike particles to vacuoles the diameter of erythrocytes and in staining characteristics from bright scarlet to deep maroon.<sup>37</sup>



Unlike the vacuoles of the monocyte, this color is not constant but changes with intravacuolar activity. The elasmatoocyte is supposed not to have mitochondria, though some have been described, obscured for the most part by the neutral red bodies. A few unstained vacuoles may not infrequently be observed at the periphery of the cell.

The *Eosinophile* is of the same size as the polynuclear cell described and is actively motile in a similar manner. Its motility, however, is not as persistent, for after a preparation has stood for some thirty or forty-five minutes at body temperature, the cell becomes globular and its nucleus clear and structureless.<sup>36, 45</sup> The cytoplasm is homogeneous and exhibits an ectoplasmic rim into which the specific granules do not obtrude. The nucleus is usually bilobate and is dragged about, passively adjusting its shape to external stress. In the active state its chromatin content is somewhat similar to that of the neutrophile. The endoplasm is filled with streaming and dancing oval granules. These granules do not vary in size or color, are much larger than the specific bodies of the poly. and have a typical bright yellow, refractile appearance.

The *Basophile* is slightly smaller than the eosinophile and likewise has ameboid motion. Its motility, however, is almost as persistent as that of the polymorphonuclear cell, continuing for several hours. Its cytoplasm is quite clear and homogeneous, and does not exhibit division into endo- and ectoplasm. Its nucleus is round or lobate and has an irregular, shallow indented contour. Lobulation is often obscured by the tendency of the lobes to fold upon each other.<sup>6</sup> They do not stretch apart as do the nuclei of other polynuclear cells. Characteristically, when this cell is observed in the motile state the nucleus appears at its fore end, in a manner similar to the nucleus of the lymphocyte. The specific bodies of the basophile are relatively smaller and more sparse than those of the eosinophile, are round in shape, and exhibit no uniformity of size. They appear as nonrefractile, deep maroon globules which stream rapidly in the cytoplasm. Mitochondria are occasionally observed and appear as a small clump of tiny, green, coccoid granules.

The progenitor of the two latterly described cells is said to be the myeloblast. The intermediary forms, the myelocytes, develop exactly as do those of the neutrophilic series, except for the fact that the specific granules are similar to those of their respective adult forms.<sup>9</sup>

*Platelets* are small, faint, bodies, approximately 1.5 to 5  $\mu$  in diameter, possessing thin vague borders. Their contour is inconstant in that it may be globular, spicular, star-shaped, or grossly irregular. The spicular configuration is the most common form. The bodies are not motile and do not exhibit brownian movement when vital. Frequently, a bit of cytoplasm broken from a motile leucocyte may resemble a platelet and lead to the false conclusion that innate motility exists. No nucleus is observed. The cytoplasm is hazy and takes a greenish tint. Occasionally a few granules stained pink with neutral red may be observed. More often, however, the small granules present, scattered indiscriminately, are stained pale blue with Janus green. The platelet is notoriously fragile and demonstrates a marked clumping tendency. Having once clumped or become adherent to glass surface, it will disintegrate, discharge its content, and fade. The number in these preparations is much diminished at the end of one hour.

## COMMENT

It is not the author's belief that the supravital technique may be utilized to the exclusion of the fixed smear. In combination with the examination of the fixed cells it offers further means of simplifying the identification of certain obscure cellular forms. The study of the living cell demonstrating specifically dyed structures offers additional aid in the differential diagnosis of blood diseases and appreciably broadens the bounds of hematologic research.<sup>11, 16, 40, 46</sup> It is with the belief that wider application should and could be made of this relatively simple procedure that this digest is offered. The simplicity of the method is stressed, for it entails no more difficulty, once the preliminary unfamiliarity has been eliminated, than any of the other established means of blood examination. In order to facilitate approach, a chart including the cardinal features of the cells commonly observed is appended.

## SUMMARY

1. The development of the use of the supravital dyes is outlined briefly.
2. The method of preparation of the solutions of Janus green and neutral red for routine use is described.
3. The preliminary steps in preparation of glassware, warm box, etc., and a few precautionary measures to insure perfection of blood spreads are discussed.
4. Detailed descriptions of each of the cell forms observed in normal and pathologic blood are offered.

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## A COLORIMETRIC MICRODETERMINATION OF CHLORIDES IN BLOOD AND URINE\*

T. V. LETONOFF, M.S., PHILADELPHIA, PA.

RECENTLY, colorimetric microdetermination of chloride has been made feasible for the clinical laboratory by the marked gain in sensitivity over existing methods effected by the work of Westfall, Findley, and Richards.<sup>1</sup> In their procedure, based on that of Isaacs<sup>2</sup> and Emich,<sup>3</sup> chromate ion displaced from silver chromate by chloride is determined with the aid of the intense purple red color formed with symmetrical diphenylcarbazine. As the chromate liberated is proportional to the concentration of chloride, comparison of the color with that yielded by a standard solution of chloride enables calculation of the quantity of chloride in unknown solutions.

Certain features of the technic described by these authors interfere with its application as a practical clinical method. Thus the diphenylcarbazine reagent keeps only a few hours, while the color formed by the reaction with chromate fades to some extent after only three minutes. In the present paper preparation of a stable diphenylcarbazine solution is described. Furthermore, the permanence of the color given by chromate with this solution has been increased.

The test has been simplified further by substitution of a pulverized protein precipitant, zinc borate, in place of colloidal zinc hydroxide. This new precipitant has not been employed previously in blood analysis. Its use here is advantageous, particularly because it does not adsorb anions, while filtrates remain free from added reagents.

The technic described is suitable for analysis of 0.1 c.c. of blood or plasma. Blood obtained by puncture of the finger tip may be analyzed without difficulty by this method.

### REAGENTS

Zinc borate powder is prepared by a method similar to that previously used in preparation of zinc hydroxide powder:<sup>4</sup> 34.8 gm. of powdered sodium borate,  $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$ , are dissolved by warming in 800 c.c. of water and

\*From the Laboratory of the Division of Metabolic Diseases of the Philadelphia General Hospital.

Received for publication, March 7, 1935.

cooled. To it is added, with stirring, 20 gm. of zinc acetate,  $\text{Zn}(\text{C}_2\text{H}_3\text{O}_2)_2 \cdot 2\text{H}_2\text{O}$ , in 200 c.c. of water. After five minutes the precipitate is filtered by vacuum and washed with water until the washings give no test for chloride with silver nitrate. About 1 liter of water is required as the chemicals used have contained chlorides as an impurity. The wet material is dried in air at room temperature on a filter paper or porous plate. Drying is complete when the material can be powdered readily. It is ground to a powder in a mortar and stored in a stoppered bottle.

*Silver Chromate.*<sup>2</sup>—Add slowly 200 c.c. of a 5.5 per cent solution of potassium chromate to 100 c.c. of boiling solution of 10 per cent silver nitrate. Silver chromate settles out rapidly. Drops of the chromate solution are added until there is a slight excess of chromate which gives the solution a yellow color. After cooling, the silver chromate is thoroughly washed with distilled water and finally dried on a Buchner funnel. The silver chromate is spread in a thin layer on a glass plate and dried in a desiccator over sulphuric acid in the dark. When dry, store in a brown bottle.

*Diphenylcarbazide, 0.02 per cent:* Weigh 0.100 gm. of powdered symmetrical diphenylcarbazide. Transfer to a 1 liter beaker, add 500 c.c. of ammonia free water and cover with a watch glass. Dissolve by boiling for a few minutes. Cool and bring to 500 c.c. in a graduated cylinder. Preserve in a brown bottle. It is stable for at least two months.

*Acetic Acid, 10 per cent:* Accurately diluted.

*Sodium Chloride Standard.*—Weigh accurately 500 mg. of pure dry sodium chloride. Dissolve in water and dilute to 100 c.c. in a volumetric flask.

*Working sodium chloride standard:* Pipette 5 c.c. of the sodium chloride standard solution into a 100 c.c. volumetric flask and dilute to mark. 0.2 c.c. = 0.05 mg. NaCl.

#### METHOD

Into exactly 1.9 c.c. of water in a centrifuge tube, deliver 0.1 c.c. blood, plasma, serum or cerebrospinal fluid, rinsing the pipette with the water. Carefully calibrated Folin micropipettes are recommended. Add about 0.05 gm. of zinc borate powder. Stopper, shake hard for at least one-half minute and allow to stand for two minutes. Filter through a small paper.

When 0.8 c.c. to 1 c.c. has been collected in a centrifuge tube, add an excess of silver chromate and stir for about one minute, rubbing the solid against the wall of the tube with a stirring rod. Centrifuge and pour the supernatant liquid through a small filter paper. Similarly treat about 1 c.c. of the working standard sodium chloride with silver chromate. Centrifuge and filter. Use 0.2 c.c. of filtrate for a standard.

Transfer 0.2 c.c. of the chromate filtrates to clean, dry test tubes. Add 3 c.c. of the 10 per cent acetic acid. Add 10 c.c. of the 0.02 per cent diphenylcarbazide, mix by inversion and let stand ten minutes. Place some of the solution in the left colorimeter cup and read the standard in the right cup.

*Calculation:*

$X = \text{mg. NaCl per 100 c.c. blood}$

$S = \text{reading of standard}$

When unknown is set at 10 mm.

$X = 50. S$

*Urine:* Prepare a 1 to 40 dilution of urine in water. Five hundredths cubic centimeter of urine made to a volume of 2 c.c. is sufficient for a determination, but more may be used if desired.

Shake about 2 c.c. of the diluted urine with about 0.05 gm. zinc borate powder and filter. Follow the technic outlined above.

*Calculation:*

$X = \text{gm. NaCl per 1,000 c.c. urine}$

$S = \text{reading of standard.}$

When unknown is set at 10 mm.

$X = S$

## EXPERIMENTAL

Accurately diluted sodium chloride solutions were prepared covering the range from 200 to 800 mg. per 100 c.c. from which the chloride was precipitated by silver chromate and the color developed as described. When the series was read against a standard equivalent to 500 mg. per 100 c.c., direct proportionality between color and concentration was obtained throughout the entire range.

The color reached maximum intensity in about ten minutes, remaining constant for twenty minutes before beginning to fade. It is obvious that all water should be chloride free.

Because of the many advantages of a solid protein precipitant, an attempt was made to utilize the zinc hydroxide powder previously described by the writer.<sup>4</sup> However, small amounts of chloride were removed by this reagent. Other protein precipitants with the exception of colloidal zinc hydroxide also adsorbed chlorides, failed to remove uric acid, or interfered with the reaction. Powdered zinc borate was then tested and found to function effectively as a protein precipitant. This substance was inert with respect to chloride. Filtrates prepared by means of zinc borate powder gave no test for protein, zinc, or borate. Uric acid, which reacts with silver chromate, was completely removed. The filtrates were neutral.

When tested by analyzing normal and pathologic blood, plasma, serum, and cerebrospinal fluid and comparing with the Van Slyke<sup>5</sup> method, the variation between the two procedures was not greater than  $\pm 2$  per cent. The diphenylcarbazide reaction likewise fell within these limits.

Analysis of protein-free filtrates after addition of pure sodium chloride to blood and plasma gave recoveries varying between 98 and 102 per cent.

## SUMMARY

A simplified microprocedure for colorimetric determination of chloride in blood, plasma, and urine is described. The chloride content of 0.1 c.e. of blood or 0.05 c.e. of urine may be determined.

Powdered zinc borate is an effective precipitant of blood proteins that does not adsorb chloride.

The author wishes to extend his thanks to Dr. John G. Reinhold for assistance with the manuscript.

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## THE ROSE BENGAL TEST OF LIVER FUNCTION: PHOTELOMETRIC METHOD\*

W. P. STOWE, M.D., AND G. D. DELPRAT, M.D., SAN FRANCISCO, CALIF.

IN PREVIOUS articles<sup>1,2</sup> we have described a simple and satisfactory dye test of liver function. In the development of the photelometer by Sanford, Sheard, and Osterberg,<sup>3</sup> for the determination of color depth by the use of a photosensitive cell, it seemed that the last difficulty was overcome by the elimination of the personal equation in color matching. The following method has been evolved and used by us with satisfaction: The use of the normal plasma blank in the central cup of the photelometer corrects automatically for the yellow tint in icteric blood, heretofore difficult to match in colorimetric methods.

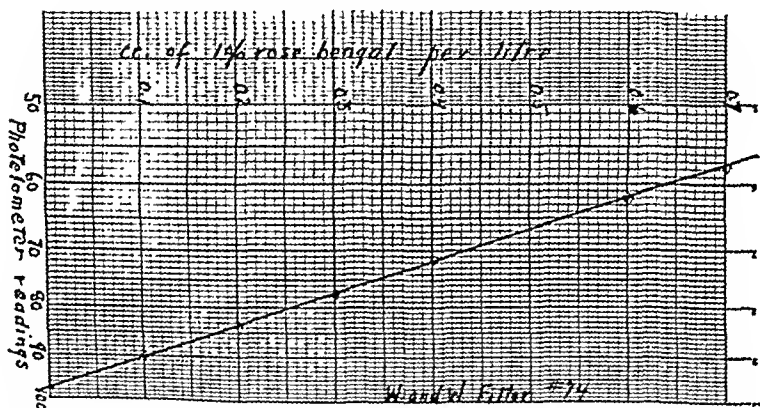


Fig. 1.—Graph for conversion of photelometer readings into parts per 1000 c.c. of solution.

The method is as follows: 10 c.c. of blood are withdrawn from an arm vein and oxalated at once (Sample A). Without withdrawing the needle, a syringe containing 10 c.c. of 1 per cent rose bengal solution in normal saline is connected and injected in about a ten-second interval. A fresh syringe filled with saline is substituted and the saline injected slowly during a two-minute interval when the second 10 c.c. blood specimen (Sample B) is removed and oxalated. Exactly six minutes from this time the third 10 c.c. specimen is removed (Sample C), either through the same needle or by separate venapuncture, and is oxalated.

All three samples are then centrifugated, the plasma removed, and the proteins precipitated with 2 c.c. of acetone per c.c. of plasma, followed by re-Na OH to clear of lipoids and bilirubinate, as previously described,<sup>2</sup> may be

\*Received for publication, April 20, 1925.



Sample A is then placed in the central cup of the photelometer with W. & W. filter No. 74 in place, and the galvanometer needle brought to 100 through this solution. Samples B and C are then read on the photelometer and the readings translated into terms of cubic centimeters of 1 per cent rose bengal solution per liter by the accompanying graph (Fig. 1), plotted on Keuffel and Esser semilogarithmic coordinate paper No. 358-51. A similar curve for any individual photelometer and filter can be easily constructed from known dilutions of the rose bengal solution used.

The calculation previously suggested,  $200 - \frac{200 \text{ Rs}}{\text{Ru}} =$  per cent of normal liver function, can be used if the parts per liter value of the two-minute sample (B) is substituted for Ru and the parts per liter value of the eight-minute sample (C) is substituted for Rs.

After extensive use we commend this method for accuracy and freedom from the errors of the "personal equation."

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ST. LUKE'S HOSPITAL.  
VALENCIA AT 27TH STREET

#### A SIMPLE BASKET CARRIER FOR USE IN TISSUE DEHYDRATION\*

ALVIN J. COX, JR., M.D., SAN FRANCISCO, CALIF.

A NUMBER of methods have been suggested for transferring tissue blocks through dehydrating fluids in preparation for embedding. In the Stanford University Department of Pathology, the use of simple wire baskets in dehydration and paraffin infiltration of routine tissues has saved much time and has nearly eliminated the possibility of loss or mislabeling of specimens. The method has been in continuous use for more than one year in the preparation of 5,000 specimens for microscopic examination.

Although a basket similar to ours may be made by a craftsman, the following method provides a cheap, rapidly made, durable basket, which can be constructed by anyone with an old pair of scissors and a pair of pliers. The materials necessary are a piece of copper window screen  $3\frac{1}{2}$  by  $2\frac{1}{2}$  inches and a smaller piece about  $1\frac{1}{4}$  inches square. Two vertical wires are removed from each end of the large piece (Fig. 1). This is then bent into approximately cylindrical shape, with the protruding ends of the horizontal wires directed inward so they interlace with those from the opposite side (Fig. 2).

\*From the Department of Pathology, Stanford University School of Medicine.  
Received for publication, April 1, 1935.

The ends of the uppermost wires are twisted about each other to hold the screen edges in position, and the joint is made more secure by weaving a piece of small copper wire back and forth as in Fig. 3. The screen is manipulated to form a cylinder, and two horizontal wires are removed from the lower end, leaving the ends of the vertical wires protruding. These are pushed through the small square piece of screen (Fig. 3). They are then bent toward the center and the protruding edges of the small piece of screen are trimmed off with scissors (Fig. 4).

The completed basket is approximately 1 inch in diameter and  $2\frac{1}{4}$  inches tall. It can be placed completely within a wide-mouthed glass-stoppered bottle 2 inches in diameter and 4 inches tall. It is conveniently lifted from one bottle to another by means of a pair of forceps. Slies of tissue up to  $\frac{7}{8}$  inch in diameter are placed in the basket, with a strip of paper bearing the

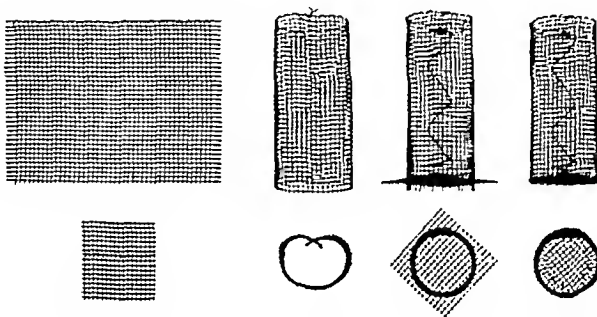


Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Figs. 1 to 4.—Stages in construction of the basket carrier. Lateral and end views.

specimen number. The tissues are not handled again until after their final immersion in melted paraffin, when they are easily removed by inverting the basket while hot. The paper strip retaining the specimen number is permanently attached to the paraffin block in which the tissues are finally embedded. This avoids errors due to faulty copying of numbers. The basket has been found to interfere very little with dehydration or paraffin infiltration if it does not contain too much tissue. One basket will carry satisfactorily 2 or 3 large pieces, or 8 to 10 pieces, of tissue.

Fresh tissues may be fixed in the baskets, which are especially valuable for fixation of tissue eurentings. These can be washed in running water without being removed. If the basket is placed in a shallow dish and the water stream directed into the top, the tissues are washed without danger of loss, since the water level within the basket does not rise above the edge of the dish.

Baskets which have been placed in melted paraffin may be cleaned easily by immersing them in waste xylene for a short period.

# DEPARTMENT OF REVIEWS AND ABSTRACTS

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ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

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## **POLIOMYELITIS, Human Convalescent Serum in the Treatment of Pre-Paralytic, Fischer, A. E. Am. J. Dis. Child. 48: 482, 1934.**

Fischer analyzed data collected on two groups of patients gathered for the purpose of determining the value of convalescent serum in the treatment of preparalytic poliomyelitis. He does not consider that the question can be answered definitely because in spite of every effort to obtain comparable data, it was not possible to do so. It has been pointed out that it is particularly difficult in the case of medical material to obtain strictly similar groups. The most important point which the study has emphasized is that 579 patients with poliomyelitis were seen early in the course of the disease, of whom 477 were treated by injection of convalescent serum and 102 were untreated. He showed that the sex, age, stage of the epidemic, day of treatment, dosage and method of administration of serum had no influence on the outcome of the disease so far as can be determined statistically, while, on the other hand, the temperature, cell count and general clinical impression could at times be used as an indication of the degree of severity of the illness.

This was the first epidemic in which an attempt was made to evaluate the serum fairly not only by treating patients in the preparalytic stage but also by examining and following a number of control patients. The author has demonstrated that the outcome for the treated patients was no better, if as good, as for the untreated ones; while the controls were probably somewhat milder, he feels that no advantage was shown by the other group. For this reason it should be possible in the future to obtain a sufficient number of control cases, for the present study has demonstrated that there is no proof that a physician is depriving his patient of an equal chance for complete recovery by not administering convalescent serum. Lacking a substitute therapeutic procedure, it would seem fair in subsequent epidemics to retest the value of potent serum in the preparalytic or meningitic stage of poliomyelitis, using, if possible, exactly parallel or alternate cases for control. If this is impossible, at least comparisons should be made as to the outcome for treated and untreated patients, using as a basis the day of the disease, the symptoms and other factors such as those discussed in this report.

## **MENINGOCOCCUS, Precipitation Reactions of, With Immune Serums in Agar Plates in Relation to Antigenic Activity, Kirkbride, M. B., and Cohen, S. M. Am. J. Hyg. 20: 444, 1934.**

Significant supplementary data on the serologic and immunologic properties and activities of the standard meningococcus strains used for many years in serum production, were obtained from studies of the precipitation reactions associated with the growth of the cultures on immune serum agar plates. At the same time, similar studies were made of a number of other strains, both recently isolated and stock. The results were, in general, in accord with previous laboratory findings which had failed to demonstrate any marked superiority in the new as compared with the older strains. Besides the laboratory observations, convincing proof of the present value of the standard strains as antigens was established by the continued favorable clinical reports on the use of the homologous therapeutic serum. Evidence was also obtained indicating the importance of maintaining the cultures used in serum production under the most favorable environmental conditions, and, conversely, the possible harmful effect on cell capacities of apparently slight alterations in these conditions and, hence, on the active principles of the serum itself. Certain of the observations made may be enumerated briefly as follows:

1. *Meningococcus* strains of Groups I and III isolated more than fifteen years previously were found to have retained their activity in elaborating the specific precipitating substance as determined by the immune serum agar plate test. These strains, also, produced, both in horses and in rabbits, serum of precipitative activity.

2. Strains of Group II and those of group "X," related to Group II or "IV," lacked, in general, demonstrable specific precipitating substance, and produced in animals serums of little or no precipitative activity.

3. Differences in the precipitation reactions of the cultures were not found to be related primarily to the interval since isolation and the period of artificial cultivation. Both stock and recent strains of Groups I and III gave rise to well-marked haloes. Neither stock nor recent strains of Group II were found to possess similar precipitative activity.

4. Substitution of certain semisolid or broth media for the dextrose serum agar used in the maintenance of cultures resulted, in the case of several Groups I and III strains, in the reduction or loss of the specific precipitating substance, and also, in the case of one strain, of the precipitinogenic properties. These results suggest that, in addition to the selection of representative strains, the medium used in their maintenance may play an important rôle in serum production.

5. Marked differences were observed in the precipitative activity of therapeutic serums produced by different laboratories.

**TYPHOID, Study of Production of Somatic and Flagellar Agglutinins in Response to Anti-typhoid-Paratyphoid Inoculations, Dennis, E. W., and Berberian, D. A. Am. J. Hyg. 20: 469, 1934.**

The serums of 114 volunteers, including individuals who had had enteric fever with and without subsequent vaccination, individuals who had no history of enterica or of vaccination, and individuals who had been vaccinated from one to ten times, were submitted to a quantitative receptor analysis before and after vaccination.

A comparative study was made of the influence of formalized, phenolized, and heat-killed vaccines prepared in our laboratory, and four commercial antityphoid preparations on the production of agglutinins.

All of the vaccines used induced the production of "O" agglutinins in considerable quantities. The vaccines prepared in the authors' laboratory of recently isolated, smooth, virulent strains gave rise to "O" titers comparable with those seen in active enteric infection; and "O" titer of 1:1,200 for *Bact. paratyphosus A* was recorded. The formalized vaccine was the most efficient in stimulating "O" agglutinins.

The vaccines prepared in their laboratory were 200 per cent more efficient than the commercial products in the production of "O" agglutinins.

There was no correlation between the number of previous vaccinations, the interval of time since the last vaccination, and the height of the titer, making the establishment of an arbitrary diagnostic titer impossible. Hence, a single qualitative receptor analysis is not capable of differentiating between inoculation agglutinins and those due to infection.

Twenty-five per cent of inoculated individuals lose all traces of "O" agglutinins within a year; more frequent inoculation is indicated.

**BILIRUBIN, BLOOD: Ernst-Foster Method for, Gadjos, A. Rev. Med-Chir. des Malad. du Foie, Paris 9: 45, 1934.**

Gadjos reports this method as superior to the van den Bergh for measuring weak bilirubinemia.

1. To 1 c.c. of serum add 2 c.c. of acetone.

2. Filter, centrifuge, and compare with standard.

The standard is a 1:6,000 solution of potassium bichromate representing 0.329 mg. per cent of bilirubin in distilled water. Comparison is made either in the colorimeter or centration is low, only 1.5 c.c. of acetone is added to the serum and the reading multiplied by 2.5.

**ANEMIA, Iron-Deficiency, Diagnosis and Treatment of, Bethell, F. H., Goldhamer, S. M., Isaacs, R., and Sturgis, C. C. J. A. M. A. 103: 797, 1934.**

Iron-deficiency anemia results from a lack of sufficient available iron for normal hemoglobin formation. Such a lack may be induced by (1) depletion of the iron reserves from continued loss of blood, (2) inadequate intake of food iron and (3) improper absorption of the element from the alimentary tract and, as a rare possibility, (4) from inability to utilize available iron.

In women with achlorhydria, anemia may develop as the result of the physiologic loss of blood. In such cases a "conditioned deficiency" dependent on the lack of hydrochloric acid may be said to exist.

The clinical features presented by patients with iron deficiency anemia are not specific. They include the effects of lack of hemoglobin supplemented by the manifestations of whatever associated condition may be present. By contrast, the blood in such patients possesses certain definite characteristics that are of diagnostic value. The relative decrease of hemoglobin exceeds that of the erythrocytes, and the average size of the red corpuscles is reduced, although proportionately to a less extent than the diminution of hemoglobin. Consequently, the color index and the mean erythrocyte diameter, volume, hemoglobin and hemoglobin concentration are below normal.

The effects of treatment with simple iron preparations of forty-two cases of iron-deficiency anemia, twenty-eight with achlorhydria, are shown in tabulated form. These results compare favorably with those reported by others employing combinations of iron with other substances in the treatment of the same type of anemia.

Relatively large amounts of ingested iron are required for satisfactory clinical and hematologic improvement. Ferrum reductum, 1.5 gm. daily, or ferrie ammonium citrate, 4 gm. daily, administered in three divided doses after meals, in our experience, is therapeutically optimal. Following the institution of treatment a latent period, during which no change in the peripheral blood picture occurs, is attributed to the time required for maturation by the primitive erythrocytes in the bone marrow. In general, the blood of patients with acid gastric secretion responds more promptly to iron medication, and a smaller dosage of the element is required than is the case of those with achlorhydria. In both groups the erythrocyte and hemoglobin values are usually restored to normal after from six to eight weeks of therapy. Patients with achlorhydria often require continued treatment with iron in order to prevent recurrence of anemia.

Administration of highly purified ferrum reductum in conjunction with a "low copper" diet do not detract from the efficacy of the iron, as evidenced by the rate of hemoglobin formation.

**HEPATIC CIRRHOSIS: The Nonglucose Reducing Bodies in Blood and Their Variation in Sugar Tolerance Tests, With Special Reference to, Menon, V. K. N., and Rao, M. V. R. Ind. J. M. Res. 22: 29, 1934.**

The nonglucose reducing bodies in blood in health and disease, with special reference to cirrhosis of the liver, were studied, and their variations in sugar tolerance tests determined. It is pointed out that:

The nonglucose reducing bodies vary within wide limits in health and disease.

The average values for "glucid X" and "y-reduction" in cases of cirrhosis of the liver do not show much variation compared to the normal standards obtained in this series. The glutathione content of the blood, however, is diminished in these cases of cirrhosis of the liver.

In most of the sugar tolerance tests in health and disease, "glucid X" and glutathione vary in the same direction as the blood sugar, while the y reduction shows a considerable fall (most marked at the end of one hour) with the rise in the blood sugar. In some cases the y-reduction actually becomes negative.

From the data at present available the nonglucose reducing bodies in blood do not seem to be in any way responsible for the low fasting blood sugar found in cases of cirrhosis of the liver.

The glutathione of the blood cannot be considered to be mainly responsible for the "γ-reduction."

A study of the ascorbic acid (vitamin C) content of the blood and its variations in sugar tolerance tests in these cases may throw further light on the nature of the "non-glucose reducing bodies" present in blood.

**SILICOSIS, Pulmonary Lesions Experimentally Produced By Intratracheal Introduction of Aluminum Oxide and Borosilicate-Glass, Lemon, W. S., and Higgins, G. M. Am. Rev. Tuberc. 30: 548, 1934.**

Very fine particles of aluminum oxide and of borosilicate-glass, of a size permitting phagocytosis, were introduced through a tracheal cannula into the lungs of two series of rabbits, and the animals were permitted to live for from one to twenty weeks.

The particles of each material produced an immediate inflammatory reaction, characterized by escape into the pulmonary tissues of serum, erythrocytes, and polymorphonuclear cells. At the same time, phagocytosis of particles occurred, and the alveolar phagocyte was the most active and important phagocytic cell. Its efficiency as a phagocyte was evidenced by the very large numbers of ingested particles. They were often so closely massed, and so numerous that they obscured the cytoplasmic detail of the cell, distorting the contour or rupturing the boundary of the cell.

Even in the experiments of shortest duration, there was evidence of migration of phagocytic cells, and of exclusion of them from the lung by way of the lymphatic structures and bronchi. The latter method of exclusion was indicated by the exudates containing phagocytic cells laden with crystals, in all of the divisions of the airway, from the alveoli themselves to the larger bronchi, the cilia of which were effective instruments in the process of removal. The former method of exclusion was indicated by finding test material in the tracheobronchial lymph nodes, and by finding evidences of agglutination of phagocytes laden with crystals in all parts of the lung where lymphatic tissue abounds.

Throughout all the experiments, the lesions which appeared as miliary or confluent, roughly spherical nodules were found in the peribronchial, perivascular and subpleural lymphatic distributions. They were conglomerate and largest when situated at the hilum, and smallest and most discrete when related to smaller bronchi or vessels.

When aluminum oxide was used, neither the histiocytes nor the fibroblasts were stimulated to activity. The lesions were made up only of aggregations of phagocytic alveolar histiocytes, which gave no evidence of multiplication nor of transformation into fibroblasts. When borosilicate glass was used, the lesions found after the longest period showed some evidence of replacement by diffuse fibrosis having taken place in the stroma of the lung. The earlier lesions were almost identical to those resulting from aluminum oxide.

There was no instance of a lesion, produced either by aluminum oxide or by borosilicate glass, that even remotely resembled those produced by silica when particles of equal size, of uncombined silicon dioxide, were similarly introduced into the lungs of rabbits. There was no evidence that either material circulated in the blood stream, and accumulation of phagocytic cells bearing crystals was not found in remote organs.

These observations seem to support the theory of solubility, of Gye and his associates, who have expressed the belief that the characteristic connective tissue nodules of silica are produced because a tissue poison results from the contact of silica with the pulmonary tissues. The authors' experiments suggest that the biologic activity of uncombined silica is absent when borosilicate glass or aluminum oxide is employed and characteristic hyperplasia does not occur.

**TUBERCULOSIS, Intestinal in 1,400 Autopsies, Crawford, P. M., and Sawyer, H. P. Am. Rev. Tuberc. 30: 568, 1934.**

In this series, 68.8 per cent of cases of fatal phthisis showed at autopsy ulcerative tuberculous lesions of the intestines.

Ulcerative intestinal tuberculosis was found as a complication of tuberculous pulmonary disease in 87.5 per cent in the colored race, as against 42.2 per cent in whites.

Tuberculous laryngitis occurred in 36.6 per cent of cases of intestinal tuberculosis, but 96.6 per cent of cases of tuberculous laryngitis showed intestinal ulceration.

Only 10.8 per cent of the ulcers in this series were of the classical girdling type.

In 540 cases of intestinal tuberculosis complicating tuberculous pulmonary disease, 53.7 per cent occurred in cases whose pulmonary symptoms had a duration of six months to three years. In 34 per cent of cases the intestinal lesions were asymptomatic, and in 35 per cent the duration of enteric symptoms was less than six months. No cases, showing preulcerative lesions only, had marked intestinal symptoms.

There were 51.6 per cent of cases classified as having had good treatment, as against 36.8 per cent of cases showing evidence of serious deficiencies in treatment.

Some form of collapse therapy for the control of the pulmonary disease had been carried out in 20 per cent of the cases.

Intestinal tuberculous ulceration is found in a majority of cases of fatal phthisis.

The portal of entry for tuberculous intestinal infection appears to be the lower ileum.

In cases of fatal phthisis with intestinal ulceration the incidence of amyloid disease is only slightly higher than in those without this complication.

A large proportion of tuberculous intestinal ulceration is not characterized by any definite symptoms during life.

The clinical detection of late tuberculous enteritis is of prognostic importance only; it portends an early fatal termination of the case.

#### **TUBERCULOSIS: Large Doses of Tuberculin in Testing Guinea Pigs Inoculated for Diagnostic Purposes, Magath, T. B. Am. J. M. Sc. 188: 403, 1934.**

When testing clinical material for the presence of *Mycobacterium tuberculosis* by animal inoculation, a pair of guinea pigs should be inoculated, one subcutaneously and the other intraperitoneally. In order to hasten the test, one or both animals should be tested at the end of four weeks with 0.5 c.c. OT tuberculin injected subcutaneously.

More than three-fourths of the positive animals will be killed within forty-eight hours and the diagnosis hastened by three weeks. The chances that any other given animal will later prove to have lesions of tuberculosis is one in thirty.

#### **EOSINOPHILIA, The Effects of Vaccines and Bacterial and Parasitic Infections On, In Trichinous Animals, Spink, W. W. Arch. Int. Med. 54: 805, 1934.**

The number of circulating eosinophile leucocytes in animals infected with *Trichinella spiralis* was reduced following infection with *B. tuberculosis*, *Staph. aureus* and *Trypanosoma equiperdum*. Animals which had received repeated injections of typhoid vaccine responded with a rise in the eosinophile level. No change was noted following the injection of heat-killed tubercle bacilli.

Studies of the bone marrows from the same animals did not reveal a corresponding decrease in the number of eosinophile cells.

Trichinous animals having a superimposed infection of tuberculosis or trypanosomiasis had less reaction around the encysted parasites in the muscles than the control animals. Trichinous animals inoculated with typhoid vaccine showed similar changes in the muscles.

Trichinous animals subjected to a high level of dry heat responded with an absolute rise in the circulating eosinophilic leucocytes.

No relationship was found between the weights of animals and the level of eosinophilic leucocytes in the peripheral blood.

The number of circulating eosinophile cells did not appear to be related to the mode of encystment of *Trichinella spiralis* in the muscle.

**HAVERHILL FEVER** (*Erythema Arthriticum Epidemicum*), Place, E. H., and Sutton, L. E. *Arch. Int. Med.* 54: 659, 1934.

An epidemic of a disease, apparently a new clinical entity, named epidemic arthritic erythema or Haverhill fever, is described. It is characterized by an abrupt onset, often with a chill, a rubellaform to morbilliform eruption, often scanty, chiefly on the extremities, with a tendency toward hemorrhage into the lesions, and an inflammation of the joints with marked pain and tenderness not infrequently of prolonged duration.

The disease occurred as a markedly localized epidemic and was undoubtedly spread through the raw milk supply.

An organism, described only once before, is believed to be the cause, having been found in the blood stream in 11 of the 17 cases in which the blood was cultured and in the fluid of the joint in the 2 cases in which the fluid was cultured.

Agglutinins were present in the blood of the infected persons but absent in the controls.

Cutaneous reactions to killed suspensions were present in 83 per cent of the patients tested late in convalescence, although absent in the controls.

Although crippling may be marked for a time, recovery tends to occur in from one to two months, with a small number of patients having persistent joint symptoms. No fatalities occurred.

**TUBERCLE BACILLUS**, Cultivation of, From the Blood Stream by Loewenstein's Method, Maier, E. *Am. Rev. Tuberc.* 30: 695, 1934.

In 198 cases of advanced pulmonary tuberculosis, attempts to cultivate the bacillus from the blood stream by the Loewenstein method gave negative results.

**LYMPHOGRANULOMA INGUINALE**, The Frei Test in, Recovery of Antigen From a Pustular Eruption, Strauss, M. J., and Howard, M. E. *J. A. M. A.* 123: 1830, 1934.

From a small series of experiments the authors conclude that:

1. If a Frei reaction is strong enough to cause vesicle formation, the contents of this vesicle are capable of producing a reaction similar to the Frei reaction in patients with lymphogranuloma inguinale.

2. The substance causing this reaction is probably a remainder of the Frei antigen originally injected and not combined with antibodies.

3. These antibodies are not circulating antibodies but fixed antibodies.

It is realized that this brief experiment is not conclusive but they feel that it is expedient to publish the results so that they may be repeated by others who have more material at their disposal and that further experiments which theirs suggest may be undertaken.



# The Journal of Laboratory and Clinical Medicine

VOL. 20

SEPTEMBER, 1935

No. 12

Editor: WARREN T. VAUGHAN, M.D.

Richmond, Va.

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## EDITORIAL

### The Treatment of Syphilis

IN AN epidemiologic study of influenza the writer in 1920 suggested the organization of an international centralized bureau for epidemiologic and similar studies of the problems of infectious disease. That others were thinking along similar lines at that time was soon evidenced by the inauguration of the Health Organization of the League of Nations.

Although the League of Nations may be impotent to cope with the problems of warfare among nations, at least one splendid advance may be credited to it, namely, its Health Organization. The situation is very similar to that accompanying the origin of the Red Cross in which doctors and scientists from all nations have succeeded reasonably well in collaboration for the good of mankind, while the nations themselves continue at each other's throats.

One of the problems which has been studied in great detail by the Organization has been that of syphilis. Much of the earlier work has been devoted

to standardization of diagnostic methods. The March, 1925, *Quarterly Bulletin* presents a discussion of the results of treatment of this disease, with collaborative effort from several countries. The countries represented are Denmark, France, Germany, Great Britain, and the United States. An idea of the standing of the collaborators in the various countries may be had from the names of those who have represented the United States: John H. Stokes, Udo J. Wile, Paul A. O'Leary, J. Earle Moore, H. N. Cole, and several members of the Public Health Service.

Conclusions drawn from the study are based on a total of 25,623 cases, approximately three-fifths men and two-fifths women. From the study, a program of ideal treatment has been elaborated, which we might term the League of Nations Health Organization Program. Since, if such a program is to be followed by the individual physician, he should know it in detail, and since this program is not generally available in the literature of this country, we herewith reproduce it in full as outlined by the Organization.

1. Treatment should be recommended as early as possible in the seronegative primary stage. In this connection, the fullest possible use should be made, for purposes of diagnosis, of the microscopic examination of secretions from primary lesions or from lymph glands.

2. It should be emphasized that, prior to the institution of either of the systems of treatment outlined below, there should be an adequate physical examination to determine the absence or otherwise of any indication for caution in respect to the dosage.

3. It is essential that, in carrying out the treatment, a strict supervision of the patient be exercised, especially in respect of mucous membranes, skin, kidneys and liver.

4. Observation, clinical and serologic, after completion of treatment should be adequate and in any case for not less than three years.

5. Adequate examination of the spinal fluid, at least before dismissal from observation, is essential.

6. The principles to be followed in carrying out the actual treatment should be as follows:

- (a) To employ a comparatively heavy individual dosage of the arsenobenzene and of the bismuth or mercurial compounds, the doses being administered in comparatively rapid succession, especially at the commencement;
- (b) To maintain a persistent attack on the disease, avoiding intervals of such length as to afford the parasite an opportunity of recovering;
- (c) To administer approximately as much treatment to primary as to secondary cases.

7. The material studied does not enable a clear decision to be made as to the relative merits of intermittent treatment with courses of injections in rapid succession separated by rest intervals of some weeks and continuous treatment as defined on pages 149 and 150 of Professor Martenstein's report, or between the simultaneous employment of both arsenical and bismuth or mercury, and the system in which bismuth and mercury are withheld until a number of arsenical injections have been administered.

Nevertheless, it seems practicable, from the results of the analysis and from the personal experience of the Experts, to formulate a system of intermittent treatment and one of continuous treatment, either of which can be expected to yield satisfactory results in ordinary cases or early syphilis.

It seems possible that the intermittent treatment which is suggested below may in effect be continuous, or practically continuous, treatment, owing to the continued absorption of bismuth from the sites of the injection for some weeks after any temporary suspension of the treatment.

## PLAN OF INTERMITTENT TREATMENT

## I. Males

For adult males of average weight aged less than 50 years and in whom there is no contraindication, a number of courses of injections on the plan described below. It should be said that, at the beginning of this course, some administer at once the full weekly dose (0.60 gm. to 0.75 gm.), while others divide it into two doses (e.g., 0.30 gm. and 0.45 gm.) so far as the first week is concerned.

WEEK	"914"	"606"	INSOLUBLE COMPOUND OF BISMUTH* CONTAINING BI-METAL
1st	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
2nd	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
3rd	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
4th	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
5th	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
6th	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
7th	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
8th	0.6 to 0.75 gm.	or 0.4 to 0.5 gm.	and 0.20 to 0.24 gm.
9th	-----	-----	0.20 to 0.24 gm.
10th	-----	-----	0.20 to 0.24 gm.

\* (1) By *insoluble bismuth* is here meant compounds of a very slight solubility in water. They should therefore be given in suspension—those of extremely slight solubility (the oxychloride, etc.) usually in a watery suspension, those that are more soluble (the subsalicylate, the iodo-bismuthate of quinine, the alkaline tartrates, etc.) suspended in a vegetable oil. If a *liposoluble* compound (e.g., the camphocarboxylate, etc.) is preferred, it is desirable that the injection be given twice weekly in half doses.

The dosage of all bismuth compounds should be calculated according to their content in bismuth metal.

(2) As an alternative to bismuth, a course of mercury may be given, either in the form of inunctions (forty days at 3 gm. of *Unguentum hydrargyri*) or of injections (70 mg. calomel or 120 mg. salicylate of mercury, etc., suspended in a suitable base).

It is recommended that:

(a) In cases which remain or become serologically negative during, or by the end of, the first course, four such courses be administered, with intervals of three to five weeks between any two courses.

(b) In cases which have not become seronegative by the end of the first course, in addition to the amount of treatment shown in (a), further courses should be administered until the patient has received as a minimum three beyond that which has ended with negative serum reactions. At the option of the individual clinician, this treatment may be prolonged as may be considered necessary.

(c) Cases presenting signs of clinical relapse of an early type should be dealt with on principles similar to those enunciated in (b).

## 2. Females

For females (nonpregnant), treatment should be administered on the plan outlined for males, with the exception that the single dose of "914" should be reduced by 0.15 gm. and that of "606" by 0.1 gm.

In the event of any reduction in the amount of treatment being indicated, it is recommended that this be effected by reducing the number of arsenical injections rather than by reducing the individual dose or increasing the interval.

As an optional scheme more in harmony with the trend toward longer courses, three series of ten to twelve injections each of the arsenical drugs may be given. To secure an overlapping of the heavy metal and the arsenical, believed by some observers to protect against neurorelapses, begin the bismuth two, three, or even four injections before the end of the longer arsenical course, continue it through the period in which the arsenical is suspended, and on into the beginning of the next arsenical course. The bismuth is then suspended while the arsenical course is completed.

# PLAN OF ALTERNATING CONTINUOUS TREATMENT FOR EARLY SYPHILIS

DAY OR WEEK	"606"	INTERIM TREATMENT	SEROL. TEST	REMARKS
Day 1	0.3-0.6	-----	1	"606" dosage for first three injections at level of 0.1 gm. for each 25 pounds (11.3 kg.) body weight. Average subsequent dosage, 0.4 gm., men; 0.3 gm., women, the fourth and subsequent injections in the first course at weekly intervals. In average patient, all lesions heal rapidly and blood serologic reaction becomes negative during first course. If "606" cannot be used, substitute 8 to 10 doses 0.3 gm. silver arsphenamine (silver salvarsan, silver arsenobenzol, etc.) or 10 to 12 doses "914" (0.45-0.6 gm. maximum for women and 0.6-0.75 gm. for men). This applies also to subsequent courses.
5	0.3-0.6	-----		
10	0.3-0.6	-----		
Week 3	0.4	-----		
4	0.4	-----		
5	0.4	-----		
6	0.4	-----		
7	.4	-----		
8	-----	Bismuth, 4 doses, 0.2 gm. and K. I., or Ungt. Hg. and K. I.	1	
9	-----	-----		
10	-----	-----		If mercury is used, note overlap of one week at end of first and start of second "606" courses. At this point, a few days without treatment may be dangerous. Neuro-relapse.
11	-----	-----		
12	0.4	-----	1	
13	0.4	-----	1	
14	0.4	-----		
15	0.4	-----		
16	0.4	-----		
17	0.4	-----		
18-23	-----	Bismuth, 6 doses (or Ungt. Hg.) and K. I.	1	
24	0.4	-----		Bismuth is better than mercury. Use it if possible. Examine cerebrospinal fluid if patient's cooperation can be secured at about this time. If found to be abnormal, continue or intensify treatment as required, re-examining fluid within six months.
25	0.4	-----		
26	0.4	-----		
27	0.4	-----		
28	0.4	-----		
29	0.4	-----		
30-37	-----	Bismuth, 8 doses (or Hg.) and K. I.	-----	
38	0.4	-----	1	
39	0.4	-----		
40	0.4	-----		
41	0.4	-----		Note that bismuth or mercury courses are gradually getting longer—four, six, eight and now ten weeks.
42	0.4	-----		
43	0.4	-----	1	
44-53	-----	Bismuth, 10 doses (or Ungt. Hg.) and K. I.	-----	
54	0.4	-----	1	
55	0.4	-----		
56	0.4	-----		
57	0.4	-----		
58	0.4	-----		
59	0.4	-----		
60-69	-----	Bismuth, 10 doses (or Ungt. Hg.) and K. I.	1	The average seronegative, seropositive primary or early secondary patient should have at least five courses of "606."
70-122	-----	Probation No treatment	6-12	
123	-----	Complete physical and neurologic examination, lumbar punctures, and, if possible, fluoroscopic examination of heart and great vessels.		

The bismuth salt advised for this system is bismuth salicylate in oil suspension, in full adult dosage with due regard for weight. Other preparations of bismuth may be used only with due regard for an equivalent metallic content and for their rate of elimination. The mercurial inunction is 50 per cent metallic mercury in a suitable fatty base, dose 4 gm. per inunction, five to six inunctions per week. *The use of the iodide is optional, depending on indications.*

The use of insoluble mercurials intramuscularly in this system is not recommended.

It should be further understood that, when heavy metal is employed after the last "606" course, the heavy metal courses are to be separated by rest intervals of six to eight weeks between each series of ten weeks' injections, or each course of forty inunctions.

In cases of primary syphilis which have remained seronegative throughout, a minimum of 5 courses of "606" or "914" should be given. Cases of seropositive primary syphilis should receive the full treatment called for by this system.

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